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# The American Journal of Medicine

VOL. III JULY, 1947 No. 1

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# The American Journal of Medicine

VOL. III JULY, 1947 No. 1

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# General Information

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*Editorial: J.A.M.A., *131*:826, July 6, 1946.

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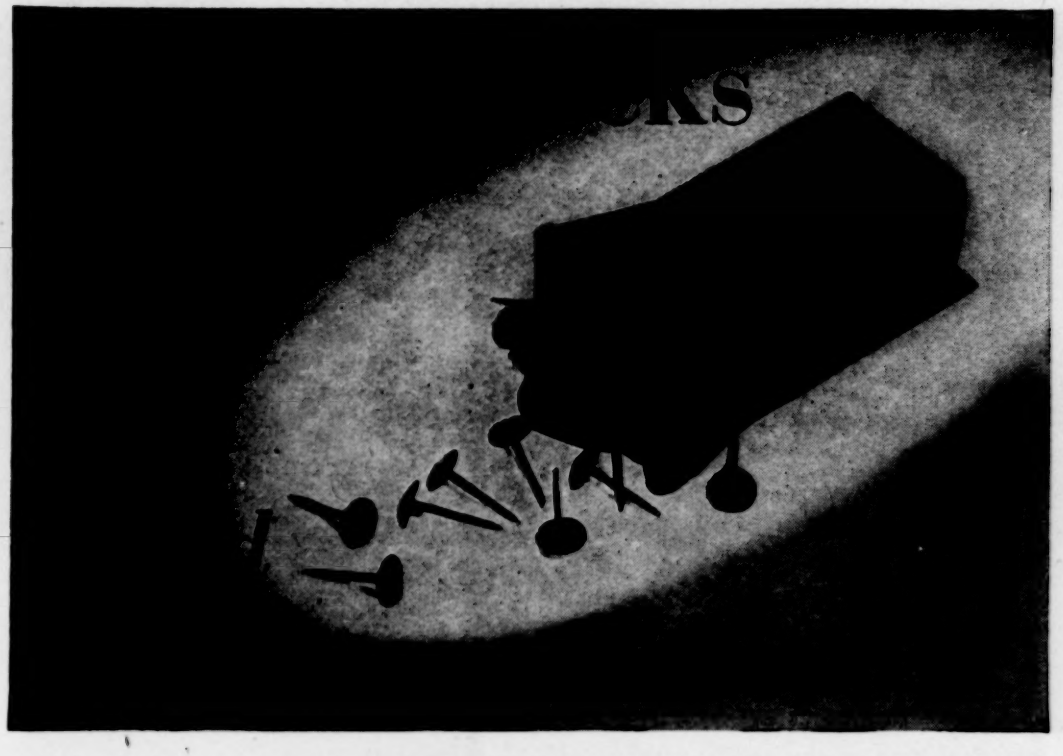

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Also, in each issue will appear Conferences on Therapy from Cornell University Medical College alternating with Combined Staff Clinics from Columbia University College of Physicians and Surgeons, New York. Both are followed by discussions. Subjects such as: Treatment of Barbiturate Poisoning, Rheumatoid Arthritis, Hypoglycemia; Treatment of Coronary Artery Disease, Nephrotic Syndrome, have already been published.

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*Diagnostic Value of Secretin Test*
Michael Lake, M.D.

*Intercapillary Glomerulosclerosis*
Lowell L. Henderson, M.D.
Randall G. Sprague, M.D.
and Henry Wagener, M.D.

*Penicillin Aerosol Therapy in Bronchiectasis, Lung abscess and Chronic Bronchitis*
Bettina Garthwaite, M.D.
and Alvan L. Barach, M.D.

*Diabetes and Hypertension*
Eugene Foldes, M.D.

**SEMINARS** — Two papers of a seminar on a particular subject are published each month until the whole seminar has been published — plus the discussions of each paper. The seminar for the first half of 1947 was on Rheumatic Fever. The last half of 1947 will deal with Thromboembolism. The 1948 issues will present Hypertension.

**SYMPOSIA**

**1**

*Present Day Status of Allergy*
by Francis M. Rackemann, M.D.
Massachusetts General Hospital, Boston
and Robert A. Cooke, M.D.
Roosevelt Hospital, N. Y.

**2**

*Aviation Medicine*
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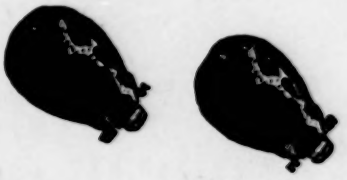

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1. J.A.M.A. 129:1160, 1945.
2. Delaware State M.J. 18:104, 1946.
3. J.A.M.A. 131:1364, 1946.
4. J.A.M.A. 132:911, 1946.
5. J.Pediat. 30:72, 1947.
6. Bol. Asoc. méd. de Puerto Rico 38: 189, 1946.
7. J.A.M.A. 131:1114, 1946.
8. Texas State J.Med. 42:314, 1946.
9. J.A.M.A. 126:349, 1944.
10. J. Lancet 67:60, 1947.
11. J.A.M.A. 131:280, 1946.
12. Lancet 2:96, 1946.


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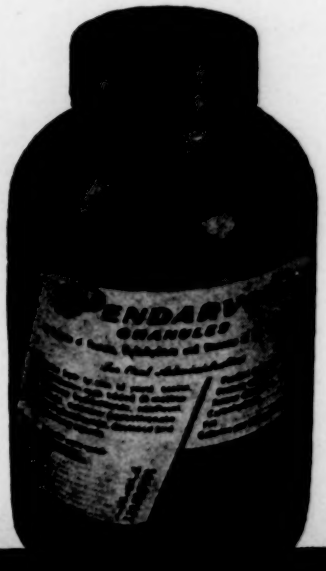

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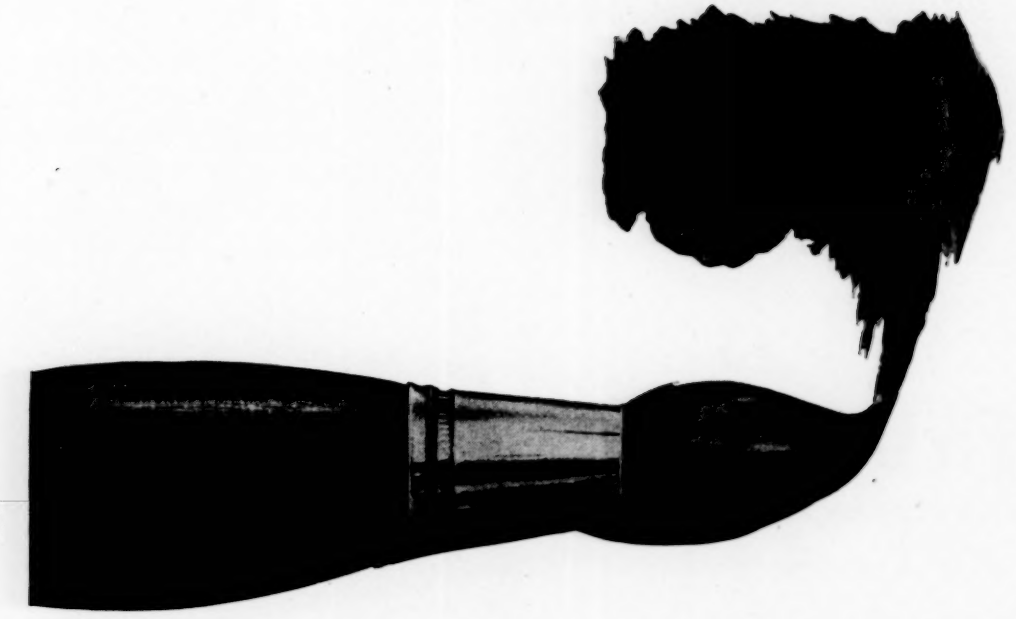

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The American Journal of Medicine

VOL. III JULY, 1947 No. 1

# Editorial

## Retrospect and Prospect

THIS issue initiates the second year of publication of The American Journal of Medicine. The occasion would seem appropriate for a review and assessment of what has been accomplished and for a statement of the program and plans for the coming year.

As set forth in the introductory editorial outlining the objectives of The American Journal of Medicine (July, 1946), it was hoped that the new journal would serve two useful and creditable functions: To add to the available media for publication of the results of sound clinical investigation; and to make more effective use of the teaching opportunities of the medical periodical as an instrument for instruction at a postgraduate level. To realize this dual aim it was proposed to combine reports of original clinical research and case studies with conferences, reviews, seminars and symposia designed chiefly for integrated teaching. By thus reflecting both the research and teaching programs of our large medical schools and clinics it was hoped to present a fairly representative cross section of current medical activities for those interested in keeping abreast of developments.

With regard to papers dealing with original investigation, it has been the policy of The American Journal of Medicine to seek a middle road between highly specialized research of immediate interest to very few and repetitious accounts of clinical experiences already familiar to most. There has been no difficulty in obtaining experimental and clinical studies of high caliber and of the desired character and scope; in fact, the influx of deserving manuscripts has been so great as to overtax the capacity of the Journal. It would appear that the Journal is already filling a real need in making available these additional facilities for publication of the results of responsible clinical investigation.

With regard to the extensive teaching program of The American Journal of Medicine, an effort has been made to choose forms of exposition that will interest and stimulate. The Conferences largely employ the time-tried Socratic method for enhancing and sustaining interest and have proved especially effective media. The Cornell Conferences on Therapy deal informatively yet informally with problems in the theory and practice of therapeutics. The topics selected are timely, the discussions are comprehensive and veered to include considerations often neglected in textbooks but which turn up (or should turn up) frequently in practice. The Columbia Combined Staff Clinics have attracted attention as an interesting development in the evolution of the clinic. Problems of disease are introduced with an extensive analysis of the basic mechanisms involved, then constructively correlated with diagnostic and therapeutic considerations to form a well rounded whole. The Washington University Clinico-pathological

Conferences have proved to be model exercises of their kind, focusing on the significance and interpretation of clinical and laboratory data rather than on statistical probabilities, yet maintaining the element of suspense. The pathological dénouement especially emphasizes such findings as might throw light upon the mechanisms of the disease under consideration.

Two interesting and instructive series of seminars have appeared, one on the therapeutic use of antibiotics, the other on rheumatic fever. A recent issue (May, 1947) contained the Journal's first symposium, a comprehensive presentation of current views on streptomycin and its therapeutic applications. Reviews and editorials summarizing a variety of appropriate subjects have been published in each issue. For all these, the seminars, symposia, reviews and editorials, it has been possible to enlist the cooperation of authoritative contributors who have given generously of their time to aid the teaching program of the Journal.

These efforts appear to have met with an appreciative response. That The American Journal of Medicine has won such wide acceptance in its first year of publication is most gratifying to the Editorial Board and to the publishers, and is taken to be an acknowledgment of the need for a journal of this kind and an endorsement of the general policies adopted. It is accordingly proposed to continue along the lines already laid down, preserving sufficient flexibility to introduce such modifications as may be indicated. The high standards in content and format will be maintained.

The program for the coming year includes a number of stimulating reports of clinical research utilizing metabolic and bacteriological technics. Controlled studies on new drugs of clinical significance will be given appropriate emphasis, one such appearing in this issue. A number of meritorious case reports are also on hand. Arrangements have been made for the continued appearance of the Conferences, which will be scheduled as in the past, the Cornell Conferences on Therapy alternating with the Columbia Combined Staff Clinics, the Washington University Clinico-pathological Conferences appearing each month.

The subjects selected for treatment as seminars (integrated series of articles appearing in six successive issues) are thromboembolism, beginning with the introductory article in this issue; and hypertension, beginning January 1948. These are timely and many-faceted topics which will be discussed from different points of view in an attempt to arrive at some clarification of their present status, particularly as to therapeutic policy. The contributors are especially qualified investigators long identified with the problems under discussion.

Another comprehensive symposium, this one on allergy under the combined guest editorships of Dr. Robert A. Cooke and Dr. Francis M. Rackemann, will appear in the fall. The symposium has been especially designed for a general medical audience. Reviews will appear in each issue, preference being given to those showing critique and constructive interpretation. A new department, "Letters to the Editor," is contemplated if there is sufficient demand; this may be utilized for brief comment on articles which have already appeared, or for early publication of abbreviated original communications.

Such are the plans for the coming year. To make possible their realization, The American Journal of Medicine looks to its many friends for continued loyal support.

ALEXANDER B. GUTMAN, M.D.

# Clinical Studies

## The Influence of Dibenamine (N, N-Dibenzyl-β-Chloroethyl-Amine) on Certain Functions of the Sympathetic Nervous System in Man*

HANS H. HECHT, M.D. *and* ROSCOE B. ANDERSON, M.D.

SALT LAKE CITY, UTAH

A SERIES of tertiary amines, structurally related to nitrogen mustards (bis- and tris-chloroethyl amines), has been recently introduced with the claim that compounds of this type block or reverse certain excitatory adrenergic responses in a variety of animals. These agents were found to be only moderately toxic and were effective when administered by mouth or vein, subcutaneously or intraperitoneally.[1] At least one chlorine and one benzyl group was found to be necessary for activity. (Fig. 1.)

One of these compounds, dibenzyl-β-chloroethyl amine ("dibenamine") was made available to us for clinical trial and has been administered as its hydrochloride salt to fifty-four patients.†

### DOSAGE AND ADMINISTRATION

Mono-chloroethyl amines are less irritant than bis- and tris-compounds (nitrogen mustards) but local irritation and tissue necrosis have been observed in animals following the administration of dibenamine and for this reason subcutaneous and intramuscular injections were avoided and no rectal suppositories were made. The compound was administered by mouth to twenty-two patients. Gelatine capsules containing 100 and 200 mg. dibenamine hydrochloride in lactose were administered with meals twice or three times daily for as long as six weeks, in doses ranging from 200 mg. to 1 Gm. per day (average 400 mg per day). Nausea, vomiting and burning in the epigastric region occurred frequently. Oral administration was abandoned when it became apparent that the pharmacologic effects in doses which could be tolerated were inconstant and unpredictable when given alone or in conjunction with parenterally administered dibenamine solutions.

Intravenous administration was found to be the only route which was safe and which gave consistent and predictable results. The effective dose which can be tolerated appears to be 4 to 6 mg./Kg. body weight (0.25 to 0.50 Gm. per patient). This was administered as a 10 per cent solution in propylene glycol or in 50 per cent acid alcohol, but it was further diluted immediately before administration to at least 50 ml. when it was given by slow injection or to 300 ml. when given by infusion. The infusion method was preferred because leakage from the vein or paravenous injection may

† Dibenamine was supplied by Givaudan-Delawanna, Inc., New York, N. Y.

* From the Department of Medicine of the University of Utah School of Medicine. Part of this investigation was supported by grants from the Fluid Research Fund of the Rockefeller Foundation, the Utah Copper Company Research Fund and the Physicians Research Fund of the University of Utah. Read, in part, at the Meeting of the Western Society for Clinical Research, San Francisco, Calif., November 1, 1946.

cause severe local reactions and since rapid intravenous injections have caused coordinated clonic convulsions in animals. As will be shown below, there is evidence that dibenamine may initiate convulsive seizures in man. Larger doses comparable to those used in animals (10 to 20 mg./Kg.) were not tolerated.

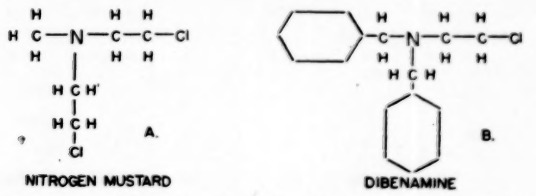


FIG. 1. A, structural formula for methyl-bis-β-chloroethyl-amine, a nitrogen mustard. B, formula for dibenzyl-β-chloroethyl amine: "Dibenamine." The structural similarity of the two compounds is apparent. The nitrogen mustards owe their physiologic activity to an intramolecular ring formation with liberation of Cl.[2] A similar mode of action has been proposed for dibenamine.[1]

## MATERIAL AND METHODS

Thirty-six patients received a total of sixty-two injections of dibenamine. Ten patients received repeated doses; in two of these, daily injections were given for six consecutive days. Toxic reactions were recorded and thirty of these patients were studied for evidence of alterations in the activity of the sympathetic nervous system. This was gauged by a number of tests which were expected to yield information on the activity of the adrenergic system at rest and during stress.

The tests may be divided into three groups. The first included those which were thought to give evidence of a blockage of the sympathetic nervous system at rest. This required observations on the reaction of the pupils to light, on changes in the temperature of the skin during and following the injection, on alterations of arterial blood pressure as an indication of a change in the resting arteriolar tone, on changes in the oscillometric indices and on alterations in sweating. Some of the undesirable reactions which occurred during the resting phase following the injection may be regarded as further evidence of an altered autonomic balance.

The second group tested the activity of the sympathetic nervous system on exercise. This was accomplished by recording the changes in arterial blood pressure upon changes in posture, upon breathing a low concentration of oxygen or carbon dioxide, upon submerging one extremity in ice water and upon breath-holding. Postural changes were checked against the "Flack test" during which the subject blows against the mercury column of a sphygmomanometer kept at 20 to 30 mm. pressure.

The third group of tests consisted of direct stimulation of the sympathetic nervous system by the injection of sympathomimetic compounds. Two were selected for trial: one was epinephrine, given intravenously in doses of 50 to 100 micrograms by syringe, or intramuscularly in doses of 10 micrograms per Kg. body weight. Before, during and after the injections, arterial and venous blood pressures, skin temperatures, heart rates and electrocardiograms were recorded and the results were analyzed and tabulated. Capillary blood sugar determinations were performed following an intramuscular injection of epinephrine. All procedures were repeated in the same subject at various intervals following an infusion of dibenamine. Neosynephrin hydrochloride was selected as a second sympathomimetic compound because, in contrast to epinephrine, its action appears to be one of peripheral vasoconstriction without appreciable direct cardiac stimulation. One mg. of neosynephrine was injected by syringe and the same procedures were carried out that were used to evaluate the epinephrine response.

It was thought that these three groups of tests performed on the same patient before

and at regular intervals after the administration of dibenamine would allow some insight into the action of this drug in man, and that the results obtained would furnish a basis for further clinical studies.

Of the thirty patients thus tested, six were suffering from essential hypertension, four from "renal" hypertension (two chronic glomerulonephritis, two intercapillary glomerulosclerosis), four had evidence of heart disease with and without cardiac irregularities, seven suffered from peripheral vascular diseases and ten were considered as normal for the purpose of this study.

### TOXIC EFFECTS

Reactions to the injection of dibenamine were frequent. Their heterogenous character may be explained by (1) the irritating nature of the drug administered (venospasm and phlebothrombosis); (2) its generally toxic effects (nausea, psychosis and convulsions) and (3) by vasodilatation (congestion of nasal mucosa and tingling of the feet). The last effects apparently result from the temporary removal of a sympathetically maintained vasomotor tone. This must be viewed as a primary action of an agent supposedly interfering with autonomic control and may not necessarily represent toxic reactions or a side effect. The same might be said for some of the reactions listed under toxic effects. These might be taken as the effects of a blockage of certain autonomic functions of the central nervous system which are at present incompletely understood. The lack of information which exists in regard to the humero-nervous regulation of many vegetative functions makes the exact evaluation of the site of action of a "sympatholytic" agent almost impossible and has prompted the listing under this heading of all effects not looked for specifically.

Such "side reactions" were always disagreeable and occurred in twenty of the thirty patients tested (67 per cent) or thirty-three times following fifty-one injections (65 per cent). Nausea with and without vomiting was frequent (twelve times in eight patients). Excessive drowsiness and dizziness were common (nine times in eight patients). Sweating, nasal congestion and tingling of the feet were noted nine times in six patients; restlessness, irritation and palpitation eight times in four patients; pain along the arms during the infusion four times in four patients and thrombophlebitis once. Mental confusion with a peculiar disturbance of time sensation, hallucinations and perseverations lasting for several hours and with full insight into the abnormal mental state during the reaction and afterwards were noted in four patients (13 per cent), but may have occurred more frequently as some of the patients appeared reluctant to recount their experiences. Once a severe convulsive seizure was noted, followed by a postconvulsive stupor lasting for several hours. The patient, who suffered from multiple sclerosis, insisted upon another injection because he "felt greatly improved." Another injection containing one-half of the original dose (5 mg./Kg.) was given two months after the first infusion; another seizure occurred but was less severe. No lasting effects were noted in any patient. No striking alterations occurred in bodily functions, including bowel habits and no changes were noted in temperature, electrocardiograms, blood counts or urinalyses.

### CERTAIN EFFECTS ON THE RESTING INDIVIDUAL

A slight reduction in systolic and diastolic arterial blood pressure over the resting preinjection value was noted seven times in twenty-seven patients. Two of these patients had resting values above the normal range. The reduction in pressure was not striking; it had disappeared within twelve hours in all but two cases. It was thought unlikely

that prolonged bed rest was the cause of these changes as these effects were transient and pre-injection values were reached within a day after the infusion had been given.

Visible peripheral vascular dilatation with flushing of the extremities was not counted for differences in measured temperatures caused by increased heat removal. (Fig. 2.)

In all patients the pupils became constricted during the infusion and became fixed within about two hours after the in-

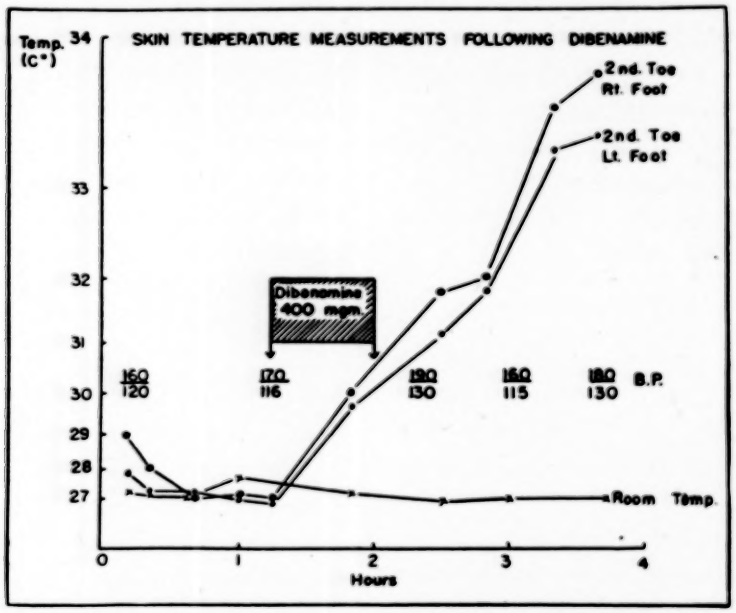


FIG. 2. Temperature measurements on two toes before, during and after a dibenamine infusion given to a male patient, thirty-five years of age, with severe essential hypertension. The patient was resting without covers in a warm room. Note the absence of blood pressure changes in spite of a rising skin temperature. The patient developed a pronounced orthostatic hypotension at the end of the test period.

common but was noted occasionally. In nine patients skin temperature measurements were performed over the distal points on upper and lower extremities at regular intervals before, during and after infusion of dibenamine. A significant increase in skin temperature was noted in four cases, all instances of peripheral vascular diseases (hypertension, thromboangiitis and Raynaud's syndrome). The changes observed were temporary, but it was considered that the measurements indicated an increase in peripheral blood flow because the temperature and humidity of the room were kept relatively constant and no alteration in sweating occurred which could have accounted for the increased heat removal.

fusion was terminated. This was a constant and long-lasting effect which gradually abated and usually disappeared by the end of the first week. The effect upon the pupils was so constant and occurred so regularly with such small doses that it was used as an indication that the agent had acted in the resting patient. No pupillary response was noted in one patient with acute glaucoma. A slight tachycardia was usually noted for several hours following the injection.

### VASCULAR RESPONSE DURING EXERCISE

When the patients rose from the supine position, a striking fall in systolic and diastolic pressures was noted in subjects

with normal blood pressure as well as in hypertensive individuals. This was commonly accompanied by dizziness and fainting and in one instance resulted in a brief convulsive seizure. As soon as the patients reclined, all symptoms disappeared and the only four were no significant changes observed (intercapillary glomerulosclerosis, Raynaud's syndrome and multiple sclerosis with extreme spasticity of the lower extremities). A precipitous drop in blood pressure was prevented by applying large

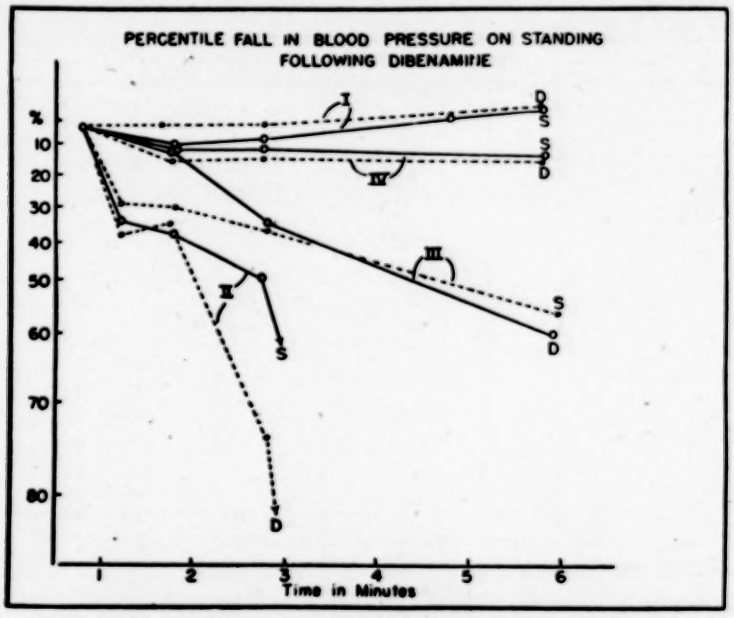


FIG. 3. Orthostatic hypotension of brief duration following a dibenamine infusion in a male patient, forty-two years of age, with severe hypertension and obliterative endarteritis. At the end of the infusion the patient fainted after standing erect for three minutes. Diastolic pressure was unobtainable at this time. I. Blood pressure before dibenamine; II. at end of infusion; III. two hours later; IV. twenty-four hours later. The changes are expressed in per cent, in relation to original blood pressure levels. S, systolic blood pressure readings; D, diastolic blood pressure readings.

arterial pressure returned to its previous level. The orthostatic hypotension thus produced was usually maximal within six hours, noticeable for one or two days and occasionally lasted longer. (Fig. 3.) In one instance, that of a far advanced case of chronic glomerulonephritis, orthostatic hypotension was noted for nine days following a single injection. In this patient no fall in blood pressure occurred on several occasions before dibenamine was administered and the hypotensive effect gradually diminished during the second week. Postural changes were tested in eighteen patients and in abdominal binders, by wrapping the lower extremities in elastic bandages or by a combination of both. (Fig. 4.)

Fall of arterial pressure upon standing may be explained in part by a loss of sensitivity of the carotid sinus.[3] It is primarily, however, a consequence of the diminished cardiac output which follows incomplete cardiac filling. This, in turn, is apparently caused by failure of postural venous constriction necessary to overcome the hydrostatic pressure within the vascular system.[4] It may be considered an instance of true forward failure with venous pooling, ap-

parently the result of an alteration of the peripheral (venous) vascular tone. This can be tested in the resting patient by raising intrathoracic pressure and substituting this for the increase in hydrostatic pressure. The intrathoracic pressure is raised by exhaling against a fixed resistance, which causes pronounced hypotension in susceptible individuals. It is the basis for the so-called Flack test which requires the patient to blow against the column of a sphygmomanometer at 20 to 30 mm. Hg pressure for 20 seconds.[4,5] A positive Flack test, indicated by a fall in arterial systolic and diastolic pressures, was always present in this series when orthostatic hypotension was noted. If for certain reasons the patient was unable to stand, a positive Flack test could be used as an indication that reflex venous constriction had been blocked. Repeated Flack tests were performed on sixteen patients, fourteen of whom were also tested for orthostatic hypotension. A negative Flack test was observed in four instances. The only discrepancy between the two tests was noted in a case of multiple sclerosis, in which spasticity of the lower extremities existed. This patient had to exert considerable muscular effort to balance herself upon standing. This may have influenced the results obtained through compensation for venous relaxation by increased muscular exertion. With the patient relaxed, a significant fall in pressures occurred during the Flack test.

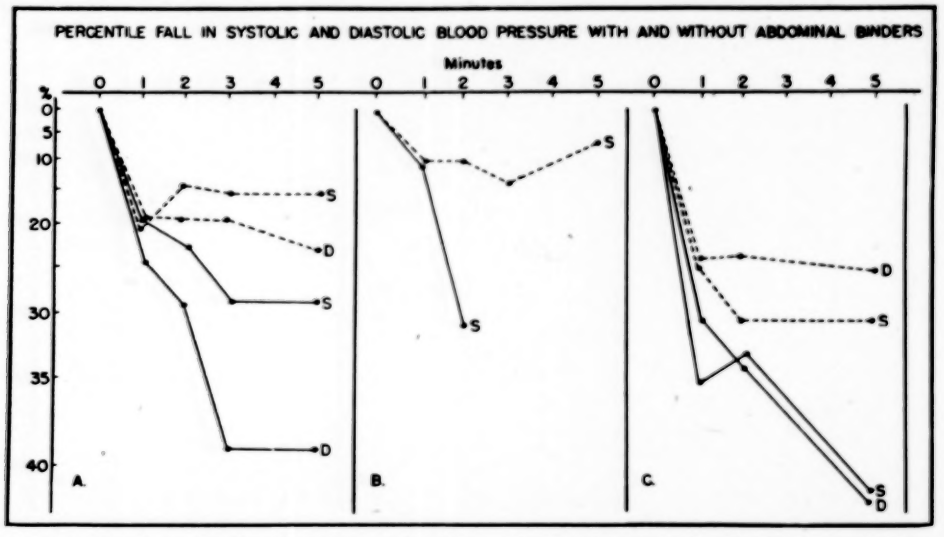


FIG. 4. Orthostatic hypotension following dibenamine infusion in three patients: A, chronic glomerulonephritis; B, essential hypertension; C, obliterative endarteritis; abdominal binders reduced the severity of the reactions. o—o systolic and •—• diastolic pressure; — without and - - - - with abdominal binders.

Arterial blood pressures will rise in response to various stimuli[6] which are largely based on reflex vasoconstriction mediated through the vasomotor centers. In the present series changes in blood pressure readings were recorded following inhalation of 10 or 12 per cent oxygen or carbon dioxide, after the patient had held his breath for 20 seconds and during and after submerging one extremity in ice water. The blood pressure response to anoxia and to inhalation of carbon dioxide was usually much less striking than that following breath-holding or subsequent to the "cold pressor test." Only the results of the latter two tests will be discussed. In the breath-holding test the patient was instructed to hold his breath for 20 seconds without forceful in- or exhalation preceding the arrest of breathing and without closure

of the glottis during the test.[7] In the eleven patients who were subjected to this procedure, no satisfactory rise was noted in six. The response in the other five individuals could be completely suppressed by administration of dibenamine, and in two individuals a slight fall in pressure was noted instead of the expected rise. The cold pressor test[8] was performed on seventeen individuals and a significant rise in pressure was noted in fifteen. Of these, a blocking of the expected response was observed in eleven individuals. No appreciable effect was noted in four (chronic glomerulonephritis, intercapillary glomerulosclerosis, achlorhydria and essential hypertension); even so a profound hypotensive effect upon change in posture could be elicited in all the subjects. Figure 5 shows the blocking effect observed in one example and Figure 6 illustrates the failure of dibenamine to block the cold pressor effect in the face of a reversal of the breath-holding test and a markedly positive Flack test lasting for three days.

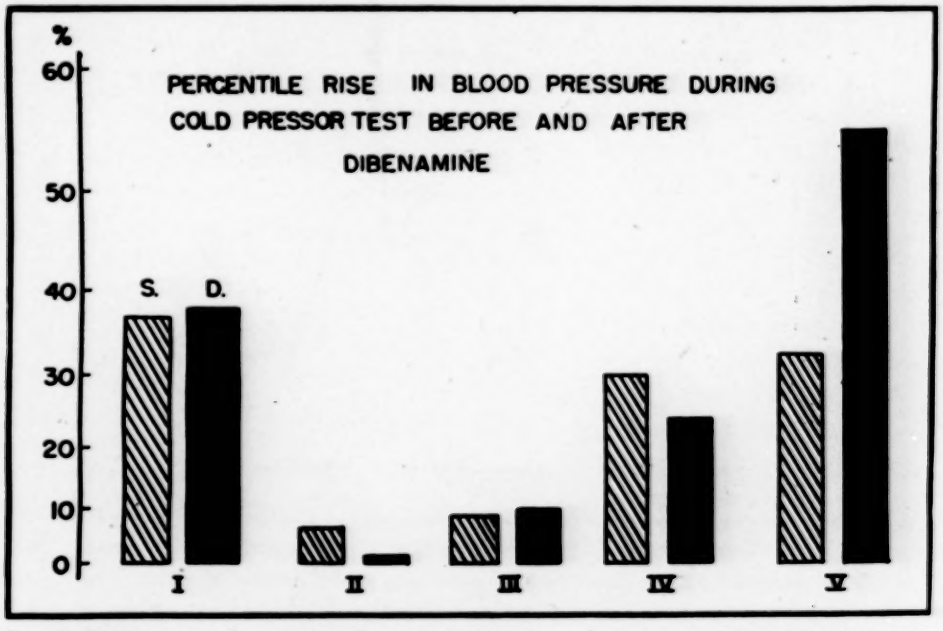


FIG. 5. Blocking of the usual reflex rise in blood pressure upon submerging one extremity in iced water for one minute (Hines' cold pressor test.)[8] The blocking effect of dibenamine was seen to last for at least two days in this forty-six year old female patient with essential hypertension and hypertensive heart disease. I. Before dibenamine; II. two hours after infusion; III, IV and V. one, two and four days, respectively, after the end of the infusion. The columns represent the percentage increase in blood pressure at one minute after onset of test. (Later values showed no qualitative differences.) S, systolic blood pressure reading; D, diastolic blood pressure reading.

## RESPONSE TO INJECTED SYMPATHOMIMETIC COMPOUNDS

*Intravenous Administration of Neosynephrin.* Previous observations have shown that the intravenous administration of this and similar compounds (paredrinol and paredrine) to normal subjects is regularly followed by a number of predictable changes; namely, a rise in systolic, diastolic and venous pressures, pounding and palpitation of the heart and excessive slowing of the heart beat.[9] A more recent analysis[10] of the changes which occur has revealed that the rise in peripheral pressure can be prevented or interrupted by vasodilator substances and that the changes in heart rate can be blocked by the administration of atropine. The

bradycardia is caused by reflex vagal slowing secondary to the rise in intravascular pressures. In man, suppression of the sinus node is followed by a variety of escape phenomena with the occurrence of auriculoventricular nodal rhythms and A.V. dissociations with and without interference and fusion beats. Prolongation of A.V. conduction is frequently seen and may be interpreted as the negative dromotropic effect of excessive vagal stimulation. Certain changes in the configuration of the ventricular deflection of the electrocardiogram are likewise explained as being secondary to vagal action. Many of these changes are peculiar to man and have been used for the experimental production of nodal rhythms and other irregularities in normal and abnormal subjects.[10] Sympathomimetic compounds of this kind appear to possess a predominant peripheral action in most individuals and cause few of the cardioexcitatory effects of epinephrine itself.

One mg. of neosynephrin was administrated by vein to nine individuals and partial or complete blocking of all these effects was readily obtained. Doses as high as 5 mg. intravenously were tolerated without any appreciable changes occurring in arterial pressure, venous pressure or in the heart rate. A summary of the changes observed after the injection of one mg. under the influence of dibenamine is given in Table I.

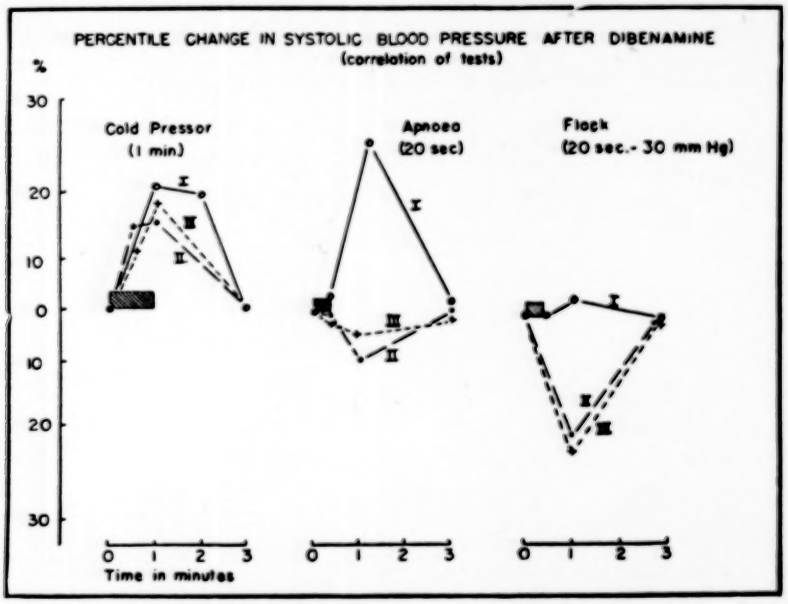


FIG. 6. A correlation of tests in a sixty-nine year old patient with portal thrombosis and a presumably normal cardiovascular system. The chart reveals that the blood pressure response to breath holding and to blowing against fixed resistance (Flack's test) was prevented by one infusion of dibenamine for at least three days, but that the cold pressor response remained essentially unaltered. Only systolic pressures are charted. I, Before dibenamine; II and II, eighteen and sixty-eight hours, respectively, after the end of the infusion.

*Intravenous Administration of Epinephrine.* The reactions following the rapid intravenous administration of small amounts of epinephrine are far more complex and quantitatively more pronounced than those observed following the injection of other sympathomimetic compounds. Briefly, the following changes can be observed when

100 micrograms (1 to 5 microgram/Kg.) are injected undiluted intravenously: Within 10 to 20 seconds, systolic, diastolic and venous pressures rise steeply, hyperventilation sets in (occasionally preceded by a short period of apnea) and the patient develops a deathly pale color due to constriction of the skin capillaries. At times pain over the lower back is experienced, severe palpitation is noted and vascular sounds and heart sounds increase in intensity. The heart rate may at first slow down in response to a vagal reflex, but it soon increases and many ectopic foci awaken and generally assume control of the heart for 1 or 2 minutes. Paroxysmal ventricular tachycardia arising from various foci and occasionally alternating in type (bidirectional) is frequent. When the effect of epinephrine on the ventricular muscle begins to wear off, auricular paroxysmal tachycardia becomes noticeable, occasionally in association with A.V. block. Although the effect has usually passed off within a few minutes, the disagreeable subjective sensations and the objective findings make this an undesirable experience both for the subject and the examiner. When an

TABLE I

PERCENTILE CHANGES OF CERTAIN VASCULAR RESPONSES TO THE INTRAVENOUS ADMINISTRATION OF 1 MG. NEOSYNEPHRIN BEFORE AND AFTER DIBENAMINE HYDROCHLORIDE (NINE PATIENTS). THE OBSERVATIONS WERE CARRIED OUT FOR TEN MINUTES

| Findings | Percentage Change | |
|---|---|---|
| | Before | After |
| | Dibenamine | |
| Average rise in systolic pressure... | 25.7 | 3.4 |
| Average rise in diastolic pressure... | 24.5 | 3.0 |
| Average rise in venous pressure (five patients)..... | 43.7 | 14.5 |
| Average decrease in heart rate..... | 30.6 | 2.5 |
| Incidence of abnormal cardiac rhythm by "sinus default"..... | 33.3 | 0.0 |

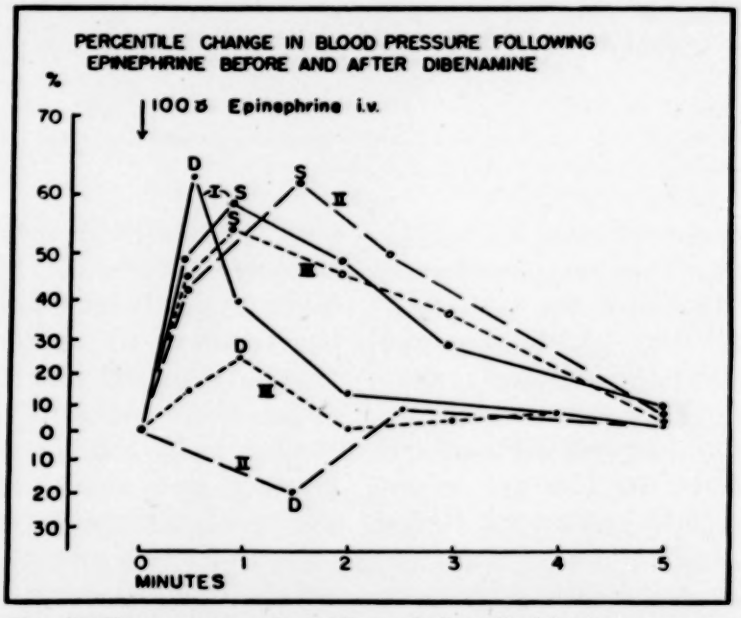


FIG. 7. The response of systolic and diastolic blood pressure to 100 micrograms epinephrine injected intravenously before and at various intervals following a dibenamine infusion (patient in Figure 6). Note that in this instance the effects of dibenamine are confined to the diastolic pressure. I, Before dibenamine; II, five hours; III, four days after an infusion of dibenamine. S, systolic pressure; D, diastolic pressure.

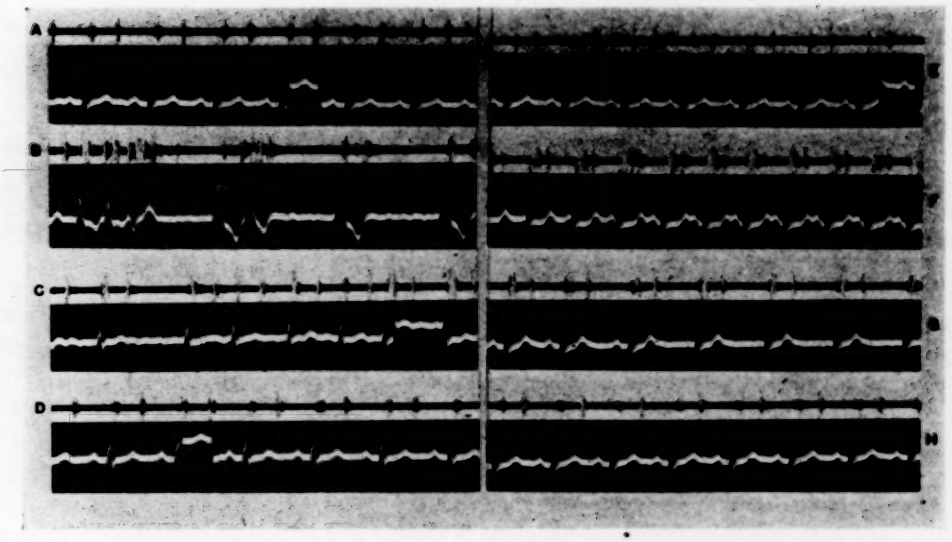

FIG. 8. Phonocardiograms from the apex and lead II of the electrocardiogram in a patient not included in this series. A and E are control records. E, two hours after an infusion of dibenamine; B and F, C and G, D and H, one, two and five minutes after the intravenous injection of 100 micrograms of epinephrine hydrochloride. In B and C a paroxysm of auricular fibrillation is seen, apparently induced by epinephrine. In B the heart is driven entirely by ectopic foci. The increase in the intensity of the first and second heart sounds and the presence of a systolic murmur may be taken as indirect indications of increased cardiac output and an increased speed of blood flow. Following dibenamine, only minor cardiac irregularities are noted (by "sinus default" due to slight increase in systolic pressure). The ventricular complexes of the electrocardiogram reveal S-T depression and in F a marked prolongation of the Q-T interval is noted. The end of the second heart sound now coincides with the rising limb of T in contrast to the usual findings seen in E and H where the end of T coincides with the end of the second sound. Note the increase in the intensity of the first and second sounds and the occurrence of a systolic murmur as before (cardiac output and circulation times unaltered by dibenamine).

attempt was made to ameliorate the effects by lowering the dose of injected epinephrine, it was noted that some of the excitatory effects did not occur and that the response more closely resembled that usually seen with neosynephrin. One hundred micrograms must therefore be given if the effects of epinephrine on the heart as well as on the peripheral vascular system are to be investigated.

The response to epinephrine given intravenously before and after the administration of dibenamine was tested in ten subjects. Following dibenamine the response was modified but was never completely blocked. The subjective sensations of the patient remained unaltered although they were less severe; hyperventilation, constriction with extreme paleness of the skin remained unchanged, the increase in intensity of the heart sounds as well as pounding and palpitation of the heart was observed and appeared similar to that experienced prior to dibenamine administration. The systolic pressure rose slightly while the diastolic pressure fell abruptly, resulting in an increase in pulse pressure over the resting values. (Fig. 7.) A fall in blood pressure following the administration of epinephrine, commonly seen in animals under the influence of various sympatholytic compounds, including dibenamine, was observed only once in an individual suffering from arteriosclerotic heart disease. In response to

epinephrine the patient developed paroxysmal tachycardia before and after dibenamine administration. A reduction of cardiac output occurred each time but was compensated by an epinephrine-induced peripheral vascular constriction before, but not after, dibenamine was given. Other examples of "partial" blood pressure reversal are shown in Figure 9. In all the subjects the heart rate increased following epinephrine and the administration of dibenamine, but the incidence of epinephrine-induced ectopic ventricular beats decreased considerably. When the one case cited above is excluded, the incidence of abnormal ventricular beats after the injection of epinephrine during one minute of continuous recording dropped from thirty-seven to one when dibenamine had been given. A summary of these findings is given in Table II.

When blocking had occurred, certain changes in the ventricular complexes of the normal heart beats, as seen in the electrocardiogram, could be observed which had been obscured by the striking irregularities present before. (Fig. 8.) Thus, a depression of the S-T segment with flattening of T previously described following the intramuscular or subcutaneous administration of epinephrine was now noted in all instances. In addition, all subjects revealed a relative prolongation of the Q-T interval (electrical systole). This appears to be a hitherto unrecognized but typical response to epinephrine which can be noted from published tables[12] and tracings.[13] In animal experiments[14,15] epinephrine shortens the mechanical systole. In man a true prolongation of the Q-T interval is present above the predicted normal, and the changes illustrated cannot be construed as indicating a shortening of the mechanical with a normal electrical systole because a true prolongation of the Q-T segment was present above the predicted normal. In this respect the disso-

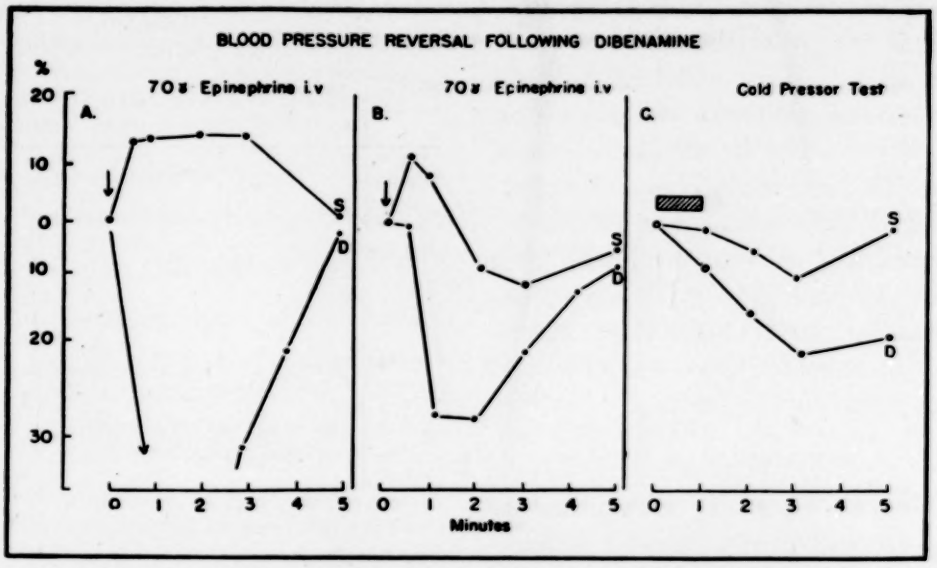


FIG. 9. "Epinephrine reversal" following dibenamine administration. A and B demonstrate a slight rise in systolic and a fall in diastolic pressures following intravenously administered epinephrine in patients previously given dibenamine. In B, a secondary fall in systolic pressure is seen. The response is similar to that seen after subcutaneous administration of epinephrine. C illustrates complete reversal of the usual blood pressure response to the "cold-pressor test." It is assumed that only the excitatory component of epinephrine (sympathin E) is blocked and that the reactions illustrated are based largely on the effects of "sympathin I," the inhibitory component.

ciation of the two events in the cardiac cycle is similar to those previously noted in hypocalcemia.[16]

Many of the effects of epinephrine appear uninfluenced by dibenamine. The present evidence indicates that cardiac output and peripheral blood flow, respiratory volume, certain effects on the capillary system and the rise in blood sugar which follow the administration of epinephrine are little influenced if preceded by the injection of dibenamine. In fact, the absence of epinephrine-induced vasoconstriction at the injection site resulted in a steeper and higher rise of blood glucose in response to epinephrine given intramuscularly after dibenamine was administered than was observed in the same individual before.

## COMMENTS

The testing of allegedly sympatholytic compounds in man is fraught with difficulties because many of the actions of the sympathetic system and the mechanisms by which they are produced have not been elucidated completely. The injection of epinephrine and related compounds cannot be considered to be a completely satisfactory mode of duplicating what happens in the intact human body upon excitation of the adrenergic system. In the present report only a few of the known mechanisms occurring spontaneously or upon the injection of such compounds have been tested. Care was taken in all instances to have the patients serve as their own controls, thus eliminating the many individual differences in response which may be obtained upon the administration of epinephrine and like substances. In this report special emphasis has been placed on the alterations occurring in the cardiovascular system because these lend themselves to simple and clear-cut experiments and the vascular system reacts so readily to small doses of epinephrine; also its response to adrenergic stimuli can be blocked easily. A tentative summary of the alterations which may be induced by dibenamine upon a number of autonomic functions is given in Table III.

No attempt is made to correlate these findings with the results observed by others using different autonomic-blocking agents or to fit these into the maze of known patterns of autonomic controls. It is possible only to speculate concerning the probable mode of action of dibenamine itself. It seems, however, that a number of clear cut changes occur which lend themselves to interpretation:

1. Little change is noted in the resting normal individual, apparently because bodily functions at rest proceed automatically and without excessive nervous control. Wherever and whenever a delicate autonomic balance appears to govern a normal

TABLE II

PERCENTILE CHANGE OF CERTAIN VASCULAR RESPONSES TO THE INTRAVENOUS ADMINISTRATION OF 50 TO 100 MICROGRAMS OF EPINEPHRINE BEFORE AND AFTER DIBENAMINE HYDROCHLORIDE (TEN PATIENTS)

| Findings | Percentage Change | |
|---|---|---|
| | Before | After |
| | Dibenamine | |
| Average rise in systolic pressure during five minute period after injection | 26.5 | 11.7 |
| Average change in diastolic pressure during five minute period after injection | 7.8 | −22.9 |
| Average increase in pulse pressure during five minute period after injection | 48.0 | 70.0 |
| Average increase in heart rate during five minute period after injection | 4.7* | 28.3 |
| Number of abnormal ventricular beats during first minute period after injection | 37 | 1 |

* The heart rate decreased temporarily in five of ten cases (secondary vagal stimulation). A decline of the heart rate was never observed under dibenamine.

or abnormal resting state, blocking of the adrenergic component must result in tipping the scale in favor of the parasympathetic system. This may explain the occasional occurrence of congestion of the nasal mucosa and regularly constricted pupils following administration of dibenamine in all individuals. In certain examples of peripheral vascular disease this concept may be used in referring to an excessive "sympathetic tone" which has been released when a temporary increase in peripheral blood flow is demonstrable after the injection of dibenamine. Such changes were never observed in normal individuals. On the same basis one expects a reduction of hypertensive blood pressure levels toward a more normal range. This was demonstrated in rats made hypertensive[1] and occasionally occurred in man but was never striking. Failure to lower abnormal blood pressure levels in man may be attributed to the relatively small doses which can be administered with safety. Whether the toxic effects (nausea, convulsions and perseverations) are caused by an impaired autonomic balance in the central nervous system itself remains a matter of speculation at present.

TABLE III
EFFECT OF DIBENAMINE HYDROCHLORIDE IN MAN (5 MG/KG.)

The following excitatory effects of the adrenergic systems are:

1. Blocked:
   (1) Dark adaptation (pupillary dilatation in dim light)
   (2) Reflex rise in vascular pressures (arterial and venous)
   (3) Secondary reflex bradycardia with escape phenomena
   (4) Secondary reflex changes in ventricular complexes
2. Partially blocked or the blocking is questionable:
   (1) Resting vascular tone (arterial and venous)
   (2) Sweat secretion*
   (3) Myocardial stimulation (ectopic foci)
3. Not blocked:
   (1) Rise in cardiac output
   (2) Constriction of certain capillary regions
   (3) Epinephrine sinus tachycardia
   (4) Epinephrine changes of ventricular complexes
   (5) Increase in electrical systole
4. Increased:
   (1) Response of blood glucose to parenteral epinephrine

* Sweat secretion is regulated by the parasympathetic system although mediated through sympathetic fibers.

2. Reflex stimulation of the adrenergic system, particularly of the vascular tree but also of other systems (pupils) appears to be blocked effectively. Whenever this was not observed the changes looked for may not have been based on epinephrine mediated reflex vasoconstriction alone (cold pressor test) or organic disease may have obviated a normal response. Failure of orthostatic hypotension to occur upon the administration of dibenamine was noted when voluntary muscular contraction overcame the pooling of blood in the veins, or when occlusive vascular disease was present to such an extent that the normal elasticity of the vascular walls had been lost. It was significant that in some of the cases in which no response to dibenamine could be elicited reflex vascular relaxation of the extremities secondary to heating other parts of the body was likewise absent, and "overswing" of the temperature following cooling of a finger failed to occur. A very excessive "sympathetic tone" not readily overcome by the usual doses of dibenamine or other measures may also have been a factor in these cases.

3. The response of the resting subject to the injection of sympathomimetic compounds was always significantly altered under the influence of dibenamine, although certain isolated reactions proceeded unchanged. Compounds whose main action consists in peripheral, arterial and venous vascular constriction appear to be deprived of their effect and can be injected in large amounts without causing any change in arterial and venous pressures. The effects of these compounds upon the heart secondary to reflex vagal stimulation occasioned by the rise in blood pressure are, of course, likewise absent. (Excessive slowing of the sinus node with escape of lower centers,

"vagal" T wave changes and alterations in A.V. conduction).

The effects of injected epinephrine may be summarized by stating that the results following rapid intravenous administration are so altered as to resemble the effects usually seen after subcutaneous injection: diastolic pressure falls and systolic pressure rises, resulting in a considerable increase in pulse pressure.[11] This is the consequence of peripheral vascular dilatation and may be explained by assuming that only the excitatory effects ("sympathin E") but not the inhibitory effects ("sympathin I") are influenced by dibenamine (Fig. 9.) This is further borne out by the obvious protection afforded by dibenamine for epinephrine-induced cardiac irregularities in man and animals. Peculiarly, other excitatory effects remain apparently uninfluenced (tachycardia, cardiac output, circulation time and capillary constriction of certain skin areas). The direct effects of epinephrine upon the heart muscle undergo no alterations after dibenamine has been given (T wave changes and lengthening of the electrical systole). Experiments testing many of the other known effects of epinephrine are now in progress.

The site of action of dibenamine must remain a matter of speculation as long as the mechanism of action of the sympathetic system is not clearly understood; however, a few negative statements can be made. Obviously, dibenamine does not act peripherally as a simple vasodilator like the nitrites (pupillary effects and blocking of ectopic foci). It cannot be considered an autonomic ganglion blocking agent similar to tetraethyl ammonium[17] because it does not alter the resting autonomic tone in general and has no actions other than those specifically directed toward certain effects of the adrenergic system. An effect of dibenamine on the central nervous system appears equally unlikely because of the peculiar specificity of its action and the absence of central effects other than those regarded as toxic. A not infrequent action of blocking agents in general consists in replacing the effector substance at its site of action. This has been assumed for anti-histaminic agents (benadryl and pyribenzamine), for antidotes of the dithiol group in metallic poisoning (BAL), and for the antagonism which exists between paraminobenzoic acid and methionine to the sulfonamides. On the basis of Cannon and Rosenblueth's theory of sympathetic mediation,[17] it may be postulated that a compound is formed in the effector cell where the mediator substance released by sympathetic nerve endings (M) is combined with one of two kinds of hypothetical substances, one inhibitory (I) and one excitatory (E). The final active compound is either sympathin E (ME) or I (MI). By analogy with other blocking compounds, replacement of the hypothetical substance E by dibenamine might be postulated to result in an ineffective MD compound instead of the effective ME combination. Formation of MI resulting in inhibitory effects, including vasodilatation, would not be affected. (Fig. 9.) This would explain the interesting and highly specific action of dibenamine upon certain excitatory effects of the adrenergic system. The lack of blocking of other effects hitherto thought to be excitatory has found no explanation at present.

The very intriguing therapeutic implications which compounds of this kind may have in spite of the disappointing results observed so far need not be considered at this time.

## SUMMARY

1. Dibenamine (dibenzyl-β-chloroethylamine), a compound shown to possess certain sympatholytic properties in animals, was administered to fifty-four patients. Only the intravenous route was found to be

safe and to yield consistent results. Given orally dibenamine was poorly tolerated and its action was unpredictable.

2. The effective single intravenous dose appeared to be 4 to 6 mg./Kg. bodyweight (0.25 to 0.50 Gm. per patient). The height of the pharmacological action of dibenamine usually occurred during the first twenty-four hours. In some patients the response to standard sympathetic stimuli appeared to be changed for several days following a single injection.

3. Following the administration of dibenamine, it was seen that some of the excitatory effects of sympathin released by stimulation of the adrenergic system were altered and appeared completely blocked when tested in the resting patient, following standard exercises or upon the intravenous injection of sympathomimetic compounds. Some of the expected responses to parenterally administered epinephrine remained unchanged or appeared potentiated by dibenamine.

The authors are indebted to Dr. L. S. Goodman for advice and criticism.

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# Diagnostic Value of the Secretin Test*

## *Including a Report of Nineteen Operated or Autopsied Cases with Anatomical Studies of the Pancreas*

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ALTHOUGH Bayliss and Starling discovered secretin in 1902, it was not until 1933 that this substance was isolated in crystalline form by Hammarsten and his collaborators.[1] Later a less pure preparation, one suitable for intravenous use in man, became available commercially.† It is heat stable, free from allergens and cholecystokinine and has caused few untoward reactions. With the use of a specially constructed double barrel gastroduodenal tube and constant suction, Agren and Lagerlöf[2,3,4] succeeded in quantitatively obtaining the duodenal contents uncontaminated for the most part by gastric juice. With this technic it became possible to study the effect of a measured dose of secretin on the external secretion of the pancreas. All observers who have reported their findings agree that the test showed great promise of being a valuable diagnostic procedure. However, the number of proven normal and pathological cases studied is still relatively small, and doubt exists as to the normal range of values and clinically significant variations. Diamond and Siegel,[5,6] who reported the largest series studied in this country, obtained results almost identical with those of the original Swedish investigators. Pratt, Brugsch and Rostler[7] obtained considerably lower values in several psychoneurotic patients, and stated "to assume as has been done that such values indicate pancreatic hypofunction of clinical significance lacks justification until supported by additional evidence not yet obtainable."

The present study was completed in 1942. The test was performed forty-three times on thirty-four individuals all of whom, with one exception, had abdominal complaints. The delay in publishing these results, except for a short report of two of the cases,[8] resulted in the opportunity of reviewing the subsequent course of the patients four years later. During this interval the pancreas of eight patients had been inspected and palpated at operation, and it was examined histologically in eleven others. In twelve additional patients prolonged clinical observation left little doubt as to the diagnosis, and the results in this group are included for comparative purposes. In three patients the diagnosis is still obscure and the results are not included in this report.

### TECHNIC AND CHEMICAL METHODS

Since we wished to compare our results with those of Agren and Lagerlöf[2,4] and Diamond and his associates[5,6] the technic and chemical methods which they describe were used, with minor modifications.

After an overnight fast a specially con-

* From the New York Hospital and the Cornell University Medical College, New York, N. Y. This investigation was aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association. Mary Cooper, B. S. and Elsa Nussbaumer gave technical assistance.

† Pancreotest, Astra Chemical Company, Sweden.

structed two-barrel tube* was passed to the proper position under fluoroscopic control. Instead of the water suction pump used by others, a small electric pump was found to be more satisfactory in that it is silent and maintains a more constant negative pressure. A negative pressure of 20 to 30 mm. of mercury was found to be sufficient. With higher pressures bleeding was occasionally encountered. The duodenal contents were collected during a twenty-minute period prior to the injection of 1 clinical unit of secretin per Kg. of body weight, and in twenty-minute fractions for sixty minutes following the secretin. Each fraction was placed in a refrigerator as soon as it was collected.

The fractions were measured for volume and the following determinations were made of each fraction:

Bicarbonate concentration in milliequivalents

Hydrogen ion concentration

Concentration in units per cc. of diastase, trypsin and lipase

The total amount of bicarbonate was calculated in terms of N/10 $NaHCO_3$ and the total of each enzyme in units (2) for the sixty-minute period following the secretin injection. Since some investigators have reported their findings in values per Kg. of body weight the weights of the subjects are included in the tables.

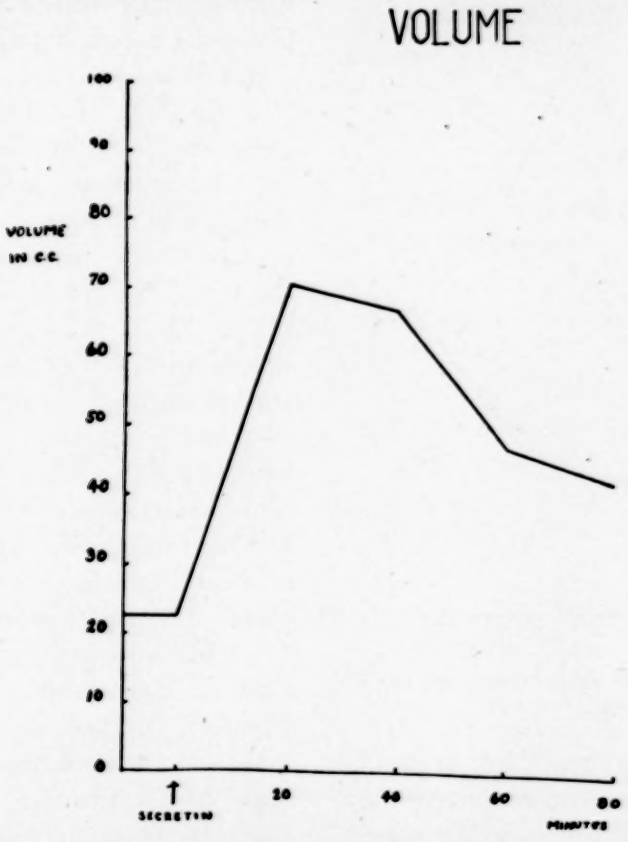


FIG. 1. Average volume per fraction.

Unfortunately, bicarbonate determinations were not made in the first nine cases of this series. When more than one test was made on the same individual the highest values obtained were given except in Case 2 in which all the values were recorded.

The method of Crandell and Cherry[16] was used for the lipase determination but its

* Davol Rubber Company.

use is not recommended as the olive oil emulsion was found to be unstable. It was noted that the values for lipase varied with the age of the olive oil emulsion. Although some of the readings were corrected by doing comparative tests with a fresh emulsion, the

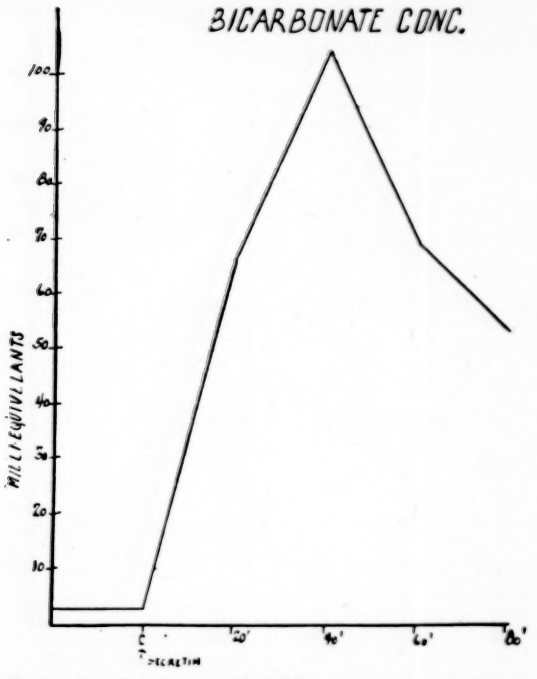


FIG. 2. Average bicarbonate concentration per fraction.

lipase values reported here are probably inaccurate to some extent, although they are comparable to values reported by others using the same method.[5,6] After this study was completed Lagerlöf[15] advised against the use of this method. He also pointed out the advisability of adding glycerin to the collecting bottles and of keeping them chilled to prevent inactivation of the trypsin during the recovery and storage of the duodenal juice. With this change in technic he obtained higher trypsin values in his more recently reported series.[15]

*Normal Material. Group I A:* The normal series consists of five cases; in two (Cases 6 and 10) the pancreas presented a normal appearance at operation, and in three (Cases 13, 32 and 36) the pancreas was examined histologically at autopsy and was found to be normal. The established diagnoses in this series were as follows:

Case 6. Gallstones
Case 10. Stones in gallbladder and common duct; obstructive jaundice
Case 13. Cirrhosis of the liver; jaundice
Case 32. Cirrhosis of the liver; jaundice
Case 36. Chronic hepatitis and splenomegaly; jaundice

*Group I B:* Twelve additional subjects, including one medical student without complaints, were sufficiently observed so that it was reasonably certain that no pancreatic disease was present at the time of the test. This group also included a patient (Case 20) with a sprue-like syndrome in whom autopsy twenty months after the test was performed showed lymphosarcoma of the small intestines with invasion of the head of the pancreas. He developed terminal jaundice. It is believed that at the time of the test the pancreas was probably normal. The clinical diagnoses are listed below:

Case 8. Anxiety neurosis
Case 14. Non-tropical sprue
Case 21. Gallstones
Case 25. Psychoneurosis
Case 27. Chronic hepatitis
Case 28. Gallstones
Case 31. Psychoneurosis
Case 33. Duodenal ulcer
Case 35. No disease
Case 20. Lymphosarcoma of small intestines with a sprue-like syndrome
Case 12. Acute hepatitis
Case 30. Common duct stone with jaundice
Case 14. Pylorospasm

*Pathological Material. Group II:* This group consists of thirteen cases with demonstrated disease of the pancreas at operation or at autopsy, as follows:

Acute pancreatic necrosis (Cases 2 and 9)
Chronic pancreatitis (Cases 11, 16, 22, 3 and 4)
Carcinoma of the tail of the pancreas (Cases 1 and 24)

Carcinoma of the head of the pancreas (Cases 3, 26 and 29)

Metastatic carcinoma of the liver, probably from the pancreas (Case 34)

*Results.* The results are presented in Tables I to IV and in Figures 1 to 3. In Tables I and II the value of each component is presented as the total for a sixty-minute period following the injection of secretin, except that the maximal concentration of bicarbonate and the highest pH obtained in this period are included. In Tables III and IV the maximum concentration of the enzymes obtained during this period are listed.

TABLE IA

PANCREAS NORMAL AT OPERATION OR AUTOPSY

| Case No. | Weight Kg. | Volume cc. | pH | Bicarbonate | | Diastase Units | Trypsin Units | Lipase Units |
|---|---|---|---|---|---|---|---|---|
| | | | | Highest Conc. | Total Output | | | |
| 6 | 58.4 | 212 | 8.2 | ... | ... | 738 | 62 | 12,835 |
| 10 | 59.5 | 345 | 7.6 | 88 | 235 | 247 | 47 | 18,400 |
| 13 | 49.3 | 177 | 8.2 | 94 | 144 | 223 | 46 | 6,534 |
| 32 | 43.3 | 94 | 8.1 | 76 | 62 | 325 | 28 | 5,987 |
| 36 | 55.5 | 199 | 8.1 | 118 | 197 | 360 | 32 | 7,735 |

TABLE IB

PANCREAS BELIEVED TO BE NORMAL

| Case No. | Weight Kg. | Volume cc. | pH | Bicarbonate | | Diastase Units | Trypsin Units | Lipase Units |
|---|---|---|---|---|---|---|---|---|
| | | | | Highest Conc. | Total Output | | | |
| 8 | 37.1 | 218 | 8.0 | ... | ... | 515 | 74 | 14,145 |
| 14 | 52 | 216 | 8.1 | 86 | 163 | 409 | 39 | 7,542 |
| 21 | 66.6 | 172 | 8.0 | 116 | 151 | 476 | 56 | 10,965 |
| 25 | 47 | 238 | 7.8 | 84 | 137 | 325 | 71 | 16,403 |
| 27 | 53.1 | 123 | 8.1 | 112 | 113 | 344 | 29 | 5,779 |
| 28 | 87.9 | 245 | 8.0 | 134 | 242 | 450 | 40 | 16,935 |
| 31 | 75 | 94 | 8.1 | 106 | 80 | 222 | 22 | 5,853 |
| 33 | 44.5 | 188 | 8.1 | 120 | 182 | 798 | 40 | 13,827 |
| 35 | 79.8 | 191 | 8.3 | 130 | 166 | 401 | 46 | 9,497 |
| 20 | 44.0 | 134 | 8.2 | 94 | 110 | 436 | 37 | 8,431 |
| 12 | 65.9 | 128 | 8.4 | 124 | 134 | 238 | 33 | 7,641 |
| 30 | 49.5 | 205 | 8.0 | 104 | 131 | 307 | 68 | 13,926 |
| 19 | 63.7 | 170 | 7.6 | 76 | 95 | 376 | 44 | 10,689 |

The range of values in the small series without pancreatic disease was as follows:

Volume ..... 94 to 345 cc.
Bicarbonate
  Maximum concentration. 76 to 134 milliequivalents
  Total in sixty minutes.... 62 to 242 N/10 $NaHCO_3$.
Diastase
  Maximum concentration. 1.26 to 8.8 units per cc.
  Total in sixty minutes.... 222 to 798 units
Trypsin
  Maximum concentration. 0.20 to 0.55 units per cc.
  Total in sixty minutes.... 22 to 74 units
Lipase
  Maximum concentration. 40.5 to 77 units per cc.
  Total in sixty minutes.... 5779 to 18,400 units

## COMMENT

*Volume.* Immediately following the injection of secretin the rate of flow of the duodenal contents rapidly increased, usually reaching its maximum in the first twenty minutes, occasionally in the second twenty minutes. The maximum twenty-

TABLE II

PANCREAS DISEASED AT OPERATION OR AUTOPSY

| Case No. | Weight Kg. | Volume cc. | pH | Bicarbonate | | Diastase Units | Trypsin Units | Lipase Units |
|---|---|---|---|---|---|---|---|---|
| | | | | Highest Conc. | Total Output | | | |
| A. Acute Pancreatitis | | | | | | | | |
| 2 a | 74.6 | 75⊥ | 8.3 | ... | ... | 153⊥ | 25 | 7,601 |
| b | 80 | 80⊥ | 8.5 | ... | ... | 76⊥ | 10⊥ | 2,486⊥ |
| c | 80.8 | 81⊥ | 8.5 | ... | ... | 119⊥ | 13⊥ | 2,763⊥ |
| 9 | 70 | 237 | 7.8 | ... | ... | 109⊥ | 49 | 9,492 |
| B. Chronic Pancreatitis | | | | | | | | |
| 11 | 39.5 | 152 | 8.2 | 104 | 149 | 195⊥ | (9.1) | 9,529 |
| 16 | 72 | 161 | 7.6 | 38⊥ | 53⊥ | 165⊥ | 40 | 8,680 |
| 22 | 64 | 187 | 7.7 | 114 | 153 | 150⊥ | 34 | 12,747 |
| 3 | 45.5 | 149 | 8.2 | ... | ... | 109⊥ | 33 | 12,778 |
| 4 | 57 | 175 | 8.1 | ... | ... | 268 | 14⊥ | 8,011 |
| C. Carcinoma of Tail of Pancreas | | | | | | | | |
| 1 | 60 | 137 | 8.1 | ... | ... | 203⊥ | 23 | 10,046 |
| 24 | 71 | 186 | 7.7 | 78 | 117 | 401 | 38 | 12,628 |
| D. Carcinoma of the Head of the Pancreas | | | | | | | | |
| 7 | 100 | 60⊥ | 8.0 | ... | ... | 21⊥ | 13⊥ | 1,917⊥ |
| 29 | 57.6 | 77⊥ | 8.1 | 64⊥ | 34⊥ | 39⊥ | 14⊥ | 1,878⊥ |
| 26 | 84 | 501⊥ | 7.8 | 96 | 382⊥ | 673⊥ | 115⊥ | 31,886⊥ |
| E. Metastatic Carcinoma of Liver, probably from the Pancreas | | | | | | | | |
| 34 | 77.8 | 130 | 8.4 | 102 | 101 | 62⊥ | 19⊥ | 5,697⊥ |

Note: Values followed by ⊥ are not within the normal range.

minute volume varied from 2.2 to nine times the presecretin volume. The average sixty-minute volume in the nonpancreatic cases was 186 cc., the minimum 94 cc. and the maximum 345 cc. In sixteen of the eighteen nonpancreatic cases the volume exceeded the minimal normal value of 135 cc. which Diamond and his co-workers[5,6] obtained. Lagerlöf[12] obtained an average volume of approximately 150 cc., a range of 104 to 266 cc. Pratt, Brugsch and Rostler,[7] when they used constant suction, obtained an average volume of 174 cc. and a lowest normal volume of 82 cc. In this study abnormally low volumes were obtained in two patients with carcinoma of the head of the pancreas (60 cc. and 77 cc.) and one case following pancreatic necrosis showed a consistently low volume in three tests (75 cc., 80 cc. and 81 cc.). Another patient with carcinoma of the pancreas with functional overactivity had the greatest volume in this series, 501 cc.

TABLE III
CASES WITHOUT PANCREATIC DISEASE
MAXIMUM CONCENTRATION OF ENZYMES

| Case No. | Diastase | Trypsin | Lipase |
|---|---|---|---|
| 6 | 6.9 | .345 | 68 |
| 10 | 1.26 | .255 | 64 |
| 13 | 1.6 | .28 | 40.5 |
| 32 | 5.6 | .465 | 62.3 |
| 36 | 8.0 | .485 | 41.5 |
| 8 | 2.9 | .435 | 65 |
| 14 | 2.4 | .20 | 41.5 |
| 21 | 3.6 | .40 | 63 |
| 25 | 2.4 | .365 | 70.5 |
| 27 | 8.8 | .55 | 49 |
| 28 | 2.8 | .30 | 73 |
| 31 | 4.8 | .425 | 66.1 |
| 33 | 8.0 | .32 | 77 |
| 35 | 3.2 | .51 | 53 |
| 20 | 4.4 | .34 | 54 |
| 12 | 3.2 | .29 | 62.8 |
| 30 | 1.7 | .36 | 72.5 |
| 19 | 2.96 | .51 | 76 |

*Bicarbonate.* Figures 2 and 3 show the average concentrations of bicarbonate and the total amount in each fraction in the cases without pancreatic disease. The maxi-

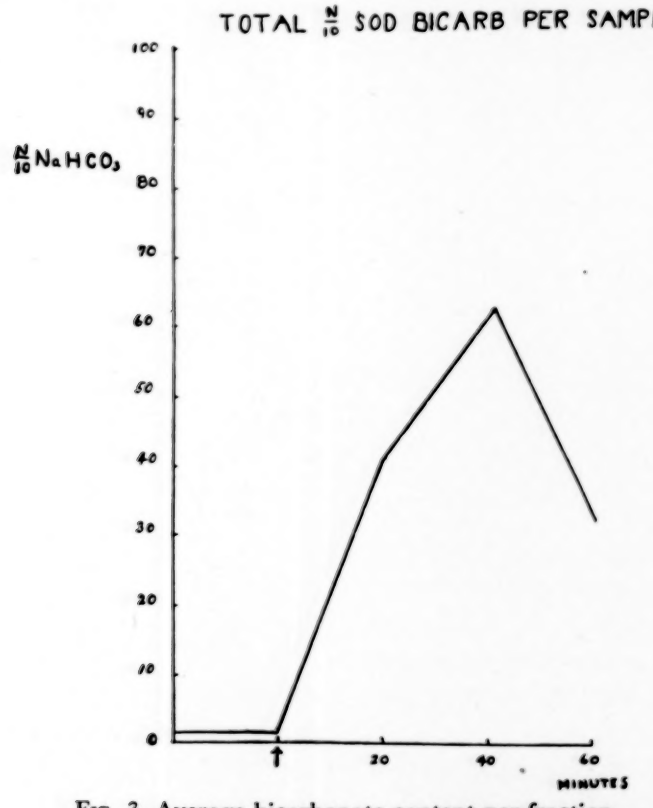


FIG. 3. Average bicarbonate content per fraction.

mum level of both is reached in the second twenty-minute period, somewhat later than the maximum volume. The pH became most alkaline at the same time, reaching 8.0 or higher in sixteen of the eighteen cases. The maximum bicarbonate concentrations in eighteen nonpancreatic cases ranged from 76 to 134 milliequivalents. Agren and Lagerlöf[4] obtained 94 to 137 milliequivalents, Diamond and Siegel[5] 90 to 130, Pollard, Miller and Brewer[10] 75 to 141, Pratt, Brugsch and Rostler[7] 50 to 115. The latter investigators, however, did not always use constant suction, which probably explains some of their low values. Five of our nonpancreatic cases had values of less than ninety. In the cases with pancreatic disease the lowest concentration was 38 milliequivalents in Case 16, and 64 milliequivalents in Case 29. The total bicarbonate secreted in sixty minutes ranged from 62 to 242, expressed as N/10 $NaHCO_3$, in our non-pancreatic cases. Only one case had less than 80. Lagerlöf[12] in forty-two normal cases obtained values of 82 to 301. Our lowest value of 34 occurred in Case 29, one with carcinoma of the pancreas. The highest value was also obtained in a case of carcinoma with hypersecretion, 382 (Case 26). Case 16, in which the biopsy showed chronic pancreatitis while the clinical course was that of carcinoma, had a total bicarbonate secretion of 53.

*Enzymes.* The concentrations of the enzymes were usually highest in the duodenal contents before the secretin injection and tended to fall as the volume of the secretion increased and to rise again in the last fraction. Occasionally, however, the concentration rose for a short time immediately following the secretin. This occurred most frequently in the case of lipase. The three enzymes often varied in concentration independently of each other. Diastase showed the greatest variability, the range in the nonpancreatic cases being from 1.26 to 8.8 units per cc. The lowest values were obtained in Case 9 (acute pancreatitis), 0.5 and in two cases of carcinoma, Case 7, 0.53 and Case 29, 0.68. In two patients with carcinoma, one with acute pancreatitis and five with chronic pancreatitis, normal values were obtained. The concentration of trypsin varied normally from 0.2 to 0.55 units per cc. and all of the pathological cases showed values within this range. Lipase concentrations varied in the nonpancreatic cases from 40.5 to 76 units per cc. Only two patients, both with car-

TABLE IV
CASES WITH PANCREATIC DISEASE
MAXIMUM CONCENTRATION OF ENZYMES

| Case No. | Diastase | Trypsin | Lipase |
|---|---|---|---|
| **Acute Pancreatitis** | | | |
| 2 a | 3.04 | .345 | 76 |
| b | 1.9 | .345 | 51 |
| c | 2.2 | .31 | 52.5 |
| 9 | 0.5⊥ | .23 | 45 |
| **Chronic Pancreatitis** | | | |
| 11 | 3.8 | .... | 71.8 |
| 16 | 2.1 | .34 | 56 |
| 22 | 1.2 | .20 | 70 |
| 3 | 1.4 | .39 | 92 |
| 4 | 2.2 | .21 | 51 |
| **Carcinoma of Tail of Pancreas** | | | |
| 1 | 1.94 | .38 | 83 |
| 24 | 5.2 | .51 | 67 |
| **Carcinoma of Head of Pancreas** | | | |
| 7 | 0.53⊥ | .21 | 33⊥ |
| 29 | 0.68⊥ | .80 | 28⊥ |
| 26 | 1.92 | .26 | 65.5 |
| **Metastatic Carcinoma of Liver, Probably from Pancreas** | | | |
| 34 | 1.4 | .26 | 54.5 |

Note: Values followed by ⊥ are below the lowest figures obtained in the normal series.

cinoma of the pancreas, showed lower values: 33 and 28 units per cc. (Cases 7 and 29). In Table IV the concentrations of the enzymes are listed for cases with pancreatic disease. It is of interest that none of our pathological cases showed a complete absence of enzymes in all fractions, since Comfort, Parker and Osterberg[9] concluded that the total absence of pancreatic enzymes alone may be taken as evidence of abnormal pancreatic function if the pancreatic enzymes are absent from more than one specimen.

The total units of enzymes obtained in sixty minutes proved to be a more frequently useful criterion of pancreatic pathology than their concentrations. In the eighteen patients without pancreatic disease, the lowest values obtained in sixty minutes were: diastase 222 units, trypsin 22 units and lipase 5,779 units. The diastase value is somewhat lower than the minimum of 300 which the Swedish investigators found in normal subjects, but considerably higher than Pratt, Brugsch and Rostler obtained. Our values for trypsin and lipase agree fairly well with those of Agren and Lagerlöf. The most frequent change noted in the cases with pancreatic disease was a decrease in diastase; this occurred in ten of the thirteen cases with pancreatic disease and was the only abnormal finding in six cases. In only one patient was the diastase value normal in the presence of any other abnormal value, such as a low trypsin value (Case 4). This seems to support Lagerlöf's statement[12] that diastase possesses the highest functional significance. Trypsin and lipase values were less easily disturbed; a decrease in both was found in one case of acute pancreatitis and in three cases of carcinoma of the pancreas.

*Bile.* Prior to the injection of secretin, bile was present in the duodenal contents. After the secretin was injected all visible evidences of bile disappeared for a variable period of time if a normally functioning gallbladder was present; otherwise, the bile color persisted throughout the test period. The mechanism by which the flow of bile is diverted to the gallbladder requires further investigation, but this response[13] proved to be a reliable test of gallbladder function in twenty-nine of thirty-one cases, a degree of accuracy comparable with Lyon's method and with the Graham test. It proved to be of particular value in a patient with acute hepatitis in whom no shadow was obtained by *x*-ray because of impaired liver function. During convalescence a normal gallbladder was visualized by *x*-ray.

## FINDINGS IN PANCREATIC DISEASE, WITH CASE REPORTS

*Acute Pancreatitis.* Two patients were tested following an operation for acute necrosis of the pancreas. Three tests were performed on one patient.

CASE 2. A forty-three year old white male chauffeur was admitted to the New York Hospital on January 6, 1941 complaining for four days of an abdominal pain. Aside from the usual childhood diseases and a fracture of the right leg eight years before, his past history was negative except for upper abdominal discomfort after meals, which he had had for several years and which was relieved by soda and peppermint. Four days before his admission he had a similar attack. Two days before he took a large dose of castor oil. Following this he had generalized abdominal pain, nausea and vomiting.

Physical examination showed an acutely ill, well nourished patient in acute abdominal distress, with a rapid pulse and tenderness of the abdomen which was most marked in the mid-epigastrium where there was extreme tenderness and spasm. A flat plate of the abdomen was negative. Following a short period of observation during which he received intravenous fluids and a blood transfusion, he was explored.

Exploration showed the presence of a large amount of rusty, cloudy fluid in the peritoneal cavity and a moderately swollen pancreas which

was studded with small, gray necrotic areas containing thick, grayish-yellow fluid. Cultures of this fluid showed no growth. Two drains were placed in the foramen of Winslow and two in the pancreas.

Postoperatively the patient had a rather stormy course; his temperature remaining elevated until the sixth postoperative day. It rose again on the eleventh day and a wound infection was found. When adequate drainage was established his general condition improved remarkably.

The secretin tests were performed approximately five, eleven and seventeen weeks following operation.

| Results of Secretin Tests | Feb. 11, 1941 | Mar. 25, 1941 | May 6, 1941 |
|---|---|---|---|
| Volume in sixty minutes | 75 | 80 | 81 |
| Highest pH | 8.3 | 8.5 | 8.5 |
| Diastase in sixty minutes | 153 | 76 | 119 |
| Trypsin in sixty minutes | 25 | 10 | 13 |
| Lipase in sixty minutes | 7601 | 2496 | 2763 |

These results indicate a progressive deterioration of pancreatic function. Subjectively, the patient had no complaints except that he was not regaining his strength as quickly as he had expected.

CASE 9. A sixty-two year old woman was admitted to the New York Hospital on March 19, 1941, complaining of abdominal pain of six days' duration and diarrhea for two days. Her past history was essentially negative except for belching and pain in the right upper quadrant and epigastrium, which radiated to the back and had been present for the past ten years. This came on after almost every meal which contained fatty food and was relieved by hot water and bicarbonate of soda. Six days before admission, after a light breakfast, she had severe right upper quadrant pain and vomiting. The vomiting stopped, but the pain persisted.

Physical examination showed an acutely ill patient with fever, rapid pulse, a distended bladder and tenderness in the right upper quadrant. The white blood count was 60,000 with 57 per cent mature and 27 per cent immature polymorphonuclear leukocytes. One specimen of urine contained sugar while several others were negative.

The day following admission an exploratory laparotomy revealed the presence of a brownish cloudy fluid in the peritoneal cavity which subsequently proved to be sterile. A markedly distended pancreas was found to which a large mass of omentum was adherent. Small patches of golden-yellow fat necrosis were seen in the omentum. On opening the pancreatic capsule the pancreas appeared to be completely necrotic throughout its length. Four drains were inserted and the abdomen closed. After a stormy convalescence, during which there was a prolonged discharge of necrotic slough and pus from the wound, she was discharged from the hospital on June 2, 1941.

On follow-up visits in April, 1942, and May, 1943, she had no complaints, but a large ventral hernia was present.

A secretin test seven weeks after operation gave the following results:

| | |
|---|---|
| Volume in sixty minutes | 237 cc. |
| Highest pH | 7.8 |
| Diastase in sixty minutes | 109 units |
| Trypsin in sixty minutes | 49 units |
| Lipase in sixty minutes | 9492 units |

These findings are within normal limits except for the diastase, which is less than half of the normal value.

*Chronic Pancreatitis.* Five patients with chronic pancreatitis were tested. In two (Cases 3 and 4), the pancreas was described as enlarged and indurated at operation on the biliary tract. In the other three cases histological studies of the pancreas were made and are reported in detail below. Case 11 is of particular interest, as the pancreatic juice was obtained from a pancreatic fistula unmixed with the duodenal secretion or bile. All five cases showed low values of one or more factors; four had low diastase values, one a low trypsin secretion and one a decreased amount of bicarbonate. All volumes were within normal limits.

CASE 11.* This fifty-six year old man was admitted to St. Luke's Hospital on March 31,

* I am indebted to Dr. William F. MacFee for the opportunity of studying this case.

1941, for the second time. During a previous admission in 1939 he had had epigastric pain and passed tarry stools, although an ulcer was not demonstrated by *x*-ray examination at that time. He complained of epigastric fullness and vomiting for a duration of four months. He also stated that he had lost 17 pounds during the past year and had become weak and irritable.

Physical examination was essentially negative. *X*-ray examination showed a markedly dilated stomach with deep hyperactive peristalsis and a large twenty-four-hour retention. The duodenal cap could not be filled by manipulation. Pyloric obstruction due to an ulcer was suspected.

Laboratory findings were within normal limits except for a low serum protein level (4.85 Gm. per cent) and moderate anemia. After a period of preparation a subtotal gastrectomy was performed and a posterior gastrojejunal anastomosis. A duodenal ulcer was found on the posterior wall eroding into the pancreas for about 2 cm. in diameter and 1 cm. in depth. The ulcer was left *in situ* and a drainage tube was inserted in this region. A large mesenteric node was removed and showed an area of tubercle formation with groups of epithelioid cells and giant cells of the Langerhans type.

Following the operation, a large amount of clear fluid drained out through the drainage tube. He began to pass large, foul stools and became markedly distended. Examination of this fluid showed it to be rich in pancreatic enzymes. The fluid was then returned to his gastrointestinal tract through a Levin tube. Within two days the distention and diarrhea subsided and his general condition was greatly improved. It was noted that the amount of drainage increased immediately after meals and during the night. On May 29, 1941, a symmetrical glove-type of dermatitis was noted on both hands. On May 26, 1941, the drainage fluid was collected for a twenty-minute period. The usual dose of secretin was then injected and further collections were made in fractions for sixty minutes. On May 31, 1941, he suddenly went into coma, had convulsions and died.

At autopsy, the left upper lobe of the lung showed extensive fibrosis with moderate lymphocytic infiltration. There was also a large calcified area and another area of fresh caseation. The renal tubules showed marked cloudy swelling. In the pancreas there was a well marked increase in the interlobular fibrous tissue. Sections through the sinus tract in the head of the pancreas showed it to be lined by dense fibrous connective tissue. On the surface there was a thin layer of necrotic tissue. The microscopic diagnoses were: Localized chronic pancreatitis, fibrocaseous pulmonary tuberculosis and chronic passive congestion of the lungs and liver.

The results of the secretin test are given in detail below:

| Sample | Time, min. | Volume, cc. | Sp. Gr. | pH | Bicarbonate Conc., milli-equivalents |
|---|---|---|---|---|---|
| Control | 20 | 25.0 | 1.016 | 7.48 | 34 |
| 1 | 20 | 55.5 | 1.013 | 7.80 | 82 |
| 2 | 20 | 48.0 | 1.012 | 8.20 | 104 |
| 3 | 20 | 48.0 | 1.012 | 8.20 | 104 |

The control fraction was opalescent, the first fraction slightly opalescent and the last two clear. The concentrations and amounts of enzymes were as follows:

| Sample | Diastase | | Trypsin | | Lipase | |
|---|---|---|---|---|---|---|
| | Conc. | Amt. | Conc. | Amt. | Conc. | Amt. |
| Control | 3.8 | 95.0 | .02 | 0.5 | 70.0 | 1750 |
| 1 | 1.4 | 77.7 | .08 | 4.4 | 71.8 | 3984.9 |
| 2 | 1.4 | 67.2 | .04 | 1.9 | 56.3 | 2702.4 |
| 3 | 1.04 | 49.9 | .06 | 2.8 | 59.2 | 2841.6 |
| Total in 60 minutes | | 194.8 | | 9.1 | | 9528.9 |

The total bicarbonate in sixty minutes was 149.1 N/10 $NaHCO_3$.

These findings are within normal limits except for the diastase, which is slightly lower than our normal low value of 222 units. No significance can be attached to the trypsin value since it was not activated.

Case 16. This seventy year old widow was admitted to the Memorial Hospital on March 26, 1941, complaining of weakness and loss of

weight of two months' duration and of painless jaundice, pruritus, dark urine and gray colored stools for three weeks' duration.

Physical examination showed the presence of jaundice, an enlarged liver and a separate globular mass extending to 2 inches above the umbilicus. *X*-ray studies showed a normal gastrointestinal tract. Urinalysis showed sugar present and the blood sugar level was elevated. On April 14, 1941, she was explored with the preoperative diagnosis of a carcinoma of the head of the pancreas. The operative findings were: an enormously distended gallbladder which contained twenty-six small, hard, black stones; the liver was grossly bile-stained but otherwise normal; a hard nodular mass was felt in the head of the pancreas and a wedge biopsy taken. A cholecystogastrostomy was performed. The patient was treated for diabetes and discharged May 24, 1941.

The biopsy was reported as showing interstitial pancreatitis with fat necrosis. There was no microscopic evidence of carcinoma in the sections examined.

Although the jaundice was relieved she continued to complain of weakness, frequent nausea and later of abdominal pain. Her weight loss continued and she died at home on August 19, 1941. An autopsy was not performed.

Although the microscopic diagnosis was chronic pancreatitis, the clinical course was very suggestive of carcinoma of the pancreas.

A secretin test was performed on July 9, 1941, after the cholecystogastrostomy, with the following result:

| | |
|---|---|
| Volume in sixty minutes | 161 cc. |
| Highest pH | 7.6 |
| Bicarbonate | |
| Highest concentration | 38 milliequivalents |
| Total in sixty minutes | 53 N/10 $NaHCO_3$ |
| Diastase in sixty minutes | 156 units |
| Trypsin in sixty minutes | 40 units |
| Lipase in sixty minutes | 8680 units |

The bicarbonate and diastase values are low; the others are within normal limits.

CASE 22. This case was reported previously,[8] except for the detailed autopsy findings in regard to the pancreas. The verified diagnosis was carcinoma of the common bile duct. The secretin test, we then stated, indicated "a normal response, except for a somewhat low diastase value resembling the results seen in pancreatitis." On gross examination several cysts were scattered throughout the organ. Subsequently, a microscopic study of the pancreas was reported as follows: "There is considerable fibrosis, both inter- and intralobular, but the acini and islands are in general well preserved. In a few ducts there appears to be metaplasia to a transitional epithelial lining. In others there is a papilloma-like growth of the duct epithelium. Included in the section is one of the cystic structures described grossly. This has a connective tissue wall and is lined on the inside by a single layer of cuboidal to slightly columnar epithelium resembling that of the pancreatic ducts. Adjacent to this are several cross sections of somewhat dilated and apparently partially occluded pancreatic ducts. In several places in the section there is moderate round cell infiltration." Anatomical diagnosis included "hyperplasia of branches of pancreatic duct with cyst formation, fibrosis of pancreas; metaplasia of duct epithelium, slight."

The results of the secretin test are included in Table II. The diastase was low, other factors were normal.

### CARCINOMA OF THE TAIL OF THE PANCREAS

In two patients with carcinoma of the tail of the pancreas, both proved by microscopic sections, both being inoperable, the following results were obtained:

| | Case 1 | Case 24 |
|---|---|---|
| Volume | 137 cc. | 186 cc. |
| Highest pH | 8.1 | 7.7 |
| Bicarbonate | | |
| Highest Conc. | | 78 milliequivalents |
| Total in 60 min. | | 117 N/10 $NaHCO_3$ |
| Diastase in 60 min. | 203 units | 401 units |
| Trypsin in 60 min. | 23 units | 38 units |
| Lipase in 60 min. | 10,046 units | 12,628 units |

These values are within normal limits except for a slight decrease in diastase in Case 1.

CARCINOMA OF THE HEAD OF THE PANCREAS

CASE 7. This sixty-two year old woman was admitted to New York Hospital on February 28, 1941, complaining of jaundice and itching of five weeks' duration. She had lost 55 pounds in the past two months and 100 pounds in the past year. Since the onset of jaundice she had vomited after every meal, but at no time complained of pain. Her past health had been excellent except for a cholecystectomy for gallstones twenty years before, at which time she was not jaundiced. She stated that twenty years before, following removal of her left ovary, her menstrual periods ceased and she began to gain weight, so that one year prior to admission her weight was 343 pounds. At the time of admission her weight was 244 pounds.

Physical examination showed jaundice, obesity, evidence of weight loss and mild hypertension.

Laboratory tests showed an icteric index of 100 on admission, 135 one week later. Stools were consistently negative for blood and bile. A gastrointestinal series was negative. The prothrombin time was normal.

She vomited frequently and felt nauseous most of the time.

On the eleventh day after admission an exploratory laparotomy was carried out. The common duct was found to be dilated. In the region of the head of the pancreas there was a hard nodular lesion about 6 cm. in diameter and running along the body of the pancreas there was a similar hard mass which seemed to be spreading from the mass in the head of the pancreas. This was thought to be carcinoma. The common duct was explored and nothing found but the mass noted in the head of the pancreas. A scoop was introduced and no stones were found. A T-tube was inserted in the common duct and the defect repaired.

Unfortunately, no biopsy was done. She was discharged to another hospital for terminal care on April 16, 1941 and died on August 9, 1941.

During the secretin test no bile was obtained through the tube, but blood was present in the duodenum. No increase in volume occurred after the secretin was injected. The following values were obtained:

| | |
|---|---|
| Volume | 60 cc. |
| Highest pH | 8.0 |
| Diastase in 60 min | 21 units |
| Trypsin in 60 min | 13 units |
| Lipase in 60 min | 1917 units |

These values are all considerably below normal.

CASE 26. This case was previously reported[8] as an example of an extremely active response of a carcinomatous pancreas in which, although the main pancreatic duct was occluded by the tumor, a large accessory duct was patent. At operation the tail of the pancreas, which appeared normal and constituted about one-sixth of the organ, was implanted into the open end of the jejunum and a gastroenterostomy and cholecystoenterostomy performed following resection of the tumor. The patient subsequently died of a recurrence of the tumor and autopsy confirmed the diagnosis of carcinoma of the pancreas. Two months after operation the secretin test was repeated and a definite response to secretin was obtained. Since the tube was introduced through a gastroenterostomy opening, contamination with the gastric contents probably explains the low bicarbonate values but a definite increase in volume occurred and the enzyme content was considerable. Results of the tests were as follows:

| | Pre-operative | Post-operative |
|---|---|---|
| Volume | 501 cc. | 118.5 cc. |
| Bicarbonate | | |
| Maximum concentration | 96 | 16 |
| Total amount | 382 | 6.7 |
| Diastase | | |
| Maximum concentration | 1.91 | 0.47 |
| Total units | 673 | 52 |
| Trypsin | | |
| Maximum concentration | 0.26 | 0.16 |
| Total units | 115 | 18 |
| Lipase | | |
| Maximum concentration | 65.5 | 77.5 |
| Total units | 31,886 | 7,111 |

Postoperative studies of fecal loss of fat and nitrogen on a liberal diet showed a daily fat loss of 31 Gm. and a nitrogen loss of 3.7 Gm. Although these values are definitely abnormal, the loss was considerably less than that obtained by Lake, Cornell and Harrison[14] in another patient on a similar diet with complete absence of pancreatic enzymes following resection of the pancreas head and in whom the fat loss was 70.7 Gm. and the nitrogen loss 6.5 Gm. When

12 Gm. of a potent pancreatic extract was given to this patient daily, the fecal fat loss decreased to 21 Gm. and the nitrogen excretion to 2.7 Gm.; the total weight of the stools also decreased.

CASE 29. A fifty-six year old Arabian cook was admitted to the New York Hospital on December 9, 1941, complaining of gradually increasing epigastric pain of three weeks' duration, which radiated around the left rib border to the left flank, and progressive jaundice, with clay colored stools, for eight days prior to admission.

Physical examination showed jaundice, a palpable liver edge 3 cm. below the costal margin and a markedly enlarged prostate.

Laboratory data revealed the following: The urine showed a faint trace of albumin and 4 plus bile. The stools were semi-formed and clay colored. There was slight anemia, a normal white count and an icteric index of 75. The prothrombin level was 11 per cent; bile was said to be present in the vomitus and a gastrointestinal series was negative.

On December 17, 1941, an exploratory laparotomy revealed a hard mass in the region of the head of the pancreas which seemed to extend the entire length of the organ. No tumor tissue was found outside of the pancreas. A cholecystogastrostomy was performed. Two weeks after operation bile was present in the eces and the icte ric index fell to 9.3.

On January 9, 1942, another exploration was done by another surgeon. The pancreas felt indurated throughout, but since it was uncertain whether this was a tumor or an infection, removal was not attempted. No biopsy was done. The patient was discharged on January 26, 1942, and died at home on August 4, 1942. The course of his disease strongly favors the diagnosis of carcinoma.

*Secretin Test*

| | |
|---|---|
| Volume | 77 cc. |
| Highest pH | 8.1 |
| Bicarbonate | |
| Highest | 64 |
| Total in sixty minutes | 34.4 |
| Diastase in sixty minutes | 39 units |
| Trypsin in sixty minutes | 14 units |
| Lipase in sixty minutes | 1878 units |

All the values are far below normal levels.

CASE 34. This patient was a sixty-three year old widow who was admitted to the New York Hospital on January 22, 1942, complaining of dull, boring pain in the left lumbar region for a duration of three months and jaundice for three weeks. The pain was worse at night and was relieved by lying on the left side. She had noted dark urine and light colored stools, and had lost 5 pounds in the previous month.

Physical examination showed moderate jaundice, a palpable liver edge and a firm mass under, but separate, from the liver margin. The Graham test showed no shadow. A gastrointestinal series showed a disturbed mucosal pattern in the second portion of the duodenum.

Both the feces and urine contained bile until February 6, 1942, when the bile disappeared from the feces. The icteric index was 125 on admission. The prothrombin level was 27 per cent but rose to 97 per cent after two ampules of vitamin K. Serum amylase and lipase were within normal limits.

On February 26, 1942, an exploratory laparotomy was performed. The liver was found to be invaded by a mass of whitish, hard tissue, which also occupied the whole right upper quadrant. It was impossible to tell its point of origin. A biopsy was reported as "metastatic adenocarcinoma, either from the biliary tract or pancreatic tissue." This patient died at home on March 27, 1942. No autopsy was performed. Secretin test gave the following results:

| | |
|---|---|
| Volume | 130 cc. |
| Highest pH | 8.4 |
| Bicarbonate | |
| Maximum concentration | 102 milliequivalents |
| Total output | 101 N/10 $NaHCO_3$ |
| Diastase in 60 minutes | 62 units |
| Trypsin in 60 minutes | 19 units |
| Lipase in 60 minutes | 5697 units |

The diastase was markedly decreased. The other enzymes were only slightly below normal. The volume and bicarbonate levels were normal.

## SUMMARY AND CONCLUSIONS

1. The results of the secretin test are given in eighteen subjects without pancreatic disease and in thirteen subjects with demonstrated disease of the pancreas. The findings agree with those of other investigators and indicate that the secretin

test is a valuable diagnostic procedure when extensive structural changes are present in the pancreas. The earliest and most frequent finding is a decrease in diastase secretion. This was usually the only abnormal finding in chronic pancreatitis.

2. A case of extensive carcinoma of the pancreas with functional hyperactivity is reported. After removal of approximately five-sixths of the organ, the remainder having been implanted into a loop of the jejunum, a definite response to secretin was demonstrated.

3. The composition of pancreatic juice obtained through a fistula was studied before and following the injection of secretin. The composition of this juice was very similar to that of the duodenal contents obtained through the swallowed tube following injection of secretin.

4. The external secretory function of the pancreas was studied at intervals following an attack of acute pancreatic necrosis, and the results showed progressive deterioration.

5. The disappearance of bile from the duodenal contents, following the injection of secretin, appears to be a reliable indication of the presence of a normally functioning gallbladder.

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# The Effect of Sodium Salicylate on the Acid-base Balance of the Blood*

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DURING a study of the effect of salicylate therapy in acute rheumatic fever,[1] several patients with high salicylate blood levels were observed to develop tachycardia, hyperpnea and low blood $CO_2$ content. Probably because of the similarity of such a syndrome with what is seen in diabetic acidosis, salicylates have generally been thought to produce acidosis.

In a recent paper Rapoport and Guest,[2] after reviewing the literature pointed out the confusion that exists about the blood changes produced by the salicylates and the mechanism of their action on the acid-base balance of the blood. From their own experiments they concluded that salicylates cause "a primary hyperventilation with lowering of the $CO_2$ tension in the blood, leading to an alkalotic tendency." In the present paper results are reported that were obtained in experiments performed to study the effect of sodium salicylate on the acid-base balance of the blood and the mechanism of its action.

Dogs weighing about 15 Kg. were used. They first received subcutaneously 1.5 to 2 cc. of a 2 per cent solution of morphine sulfate and then intravenously from 1.25 to 1.75 cc. per Kg. of weight of a 20 per cent aqueous solution of sodium barbital. After a period of approximately two hours, changes in respiratory rate were followed by direct observation without recording the depth of respiration. Changes in temperature were read from a rectal thermometer. Blood samples were drawn from the femoral artery to follow changes in red cell volume, pH, $O_2$ and $CO_2$ content. The red cell volume was determined by the hematocrit method while the $CO_2$ content and pH values were determined for the most part on whole blood and occasionally on plasma.

The effects of the infusion of saline solution were studied in one control experiment. The dog was anesthetized as described and received intravenously 10 cc. per Kg. of weight of an 0.85 per cent aqueous solution of sodium chloride. The time allowed for the infusion was essentially similar in the control experiment and in the salicylate experiments.

Figure 1 is a graphic representation of the control experiment. It can readily be seen that temperature and respiratory rate as well as pH, oxygen content and $CO_2$ content of the whole blood changed but little; red cell volume first decreased and then increased.

The effects of the infusion of sodium salicylate were studied in nine experiments. Doses of sodium salicylate ranging from 0.19 Gm. per Kg. to 0.6 Gm. per Kg. were administered, dissolved in distilled water or in an 0.85 per cent aqueous solution of sodium chloride or in a mixture containing half of each. The total volume of the infusion ranged from 5 cc. per Kg. to 11 cc. per Kg. The time allowed for the administration ranged between fifteen and sixty minutes. The blood changes observed in all experiments were qualitatively essentially similar.

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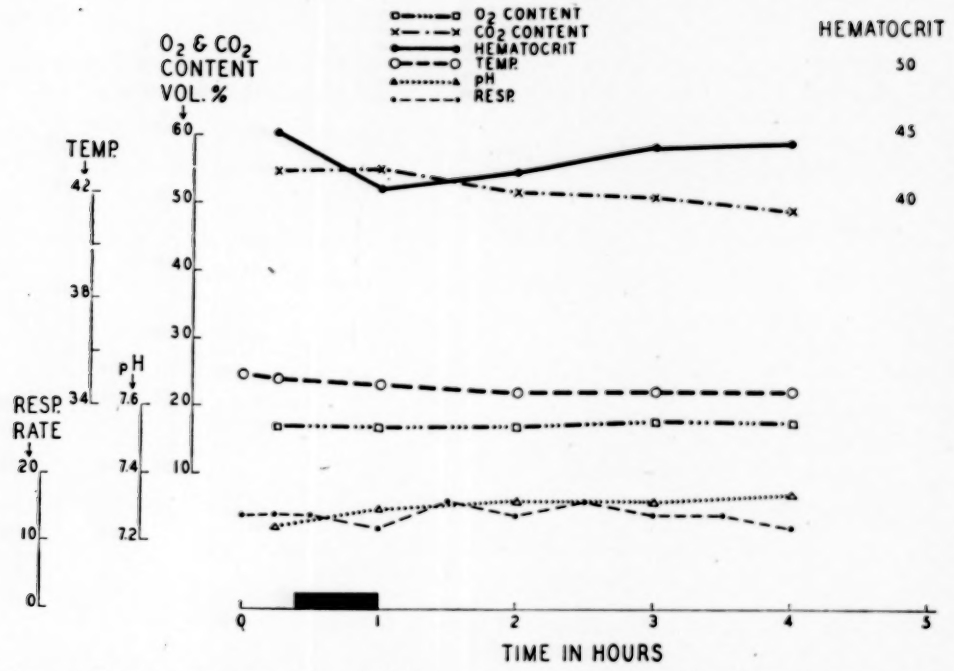


FIG. 1. Effect of the intravenous administration of 10 cc. per Kg. of an 0.85 per cent aqueous solution of sodium chloride on the acid-base balance of a dog under morphine-sodium barbital anesthesia.

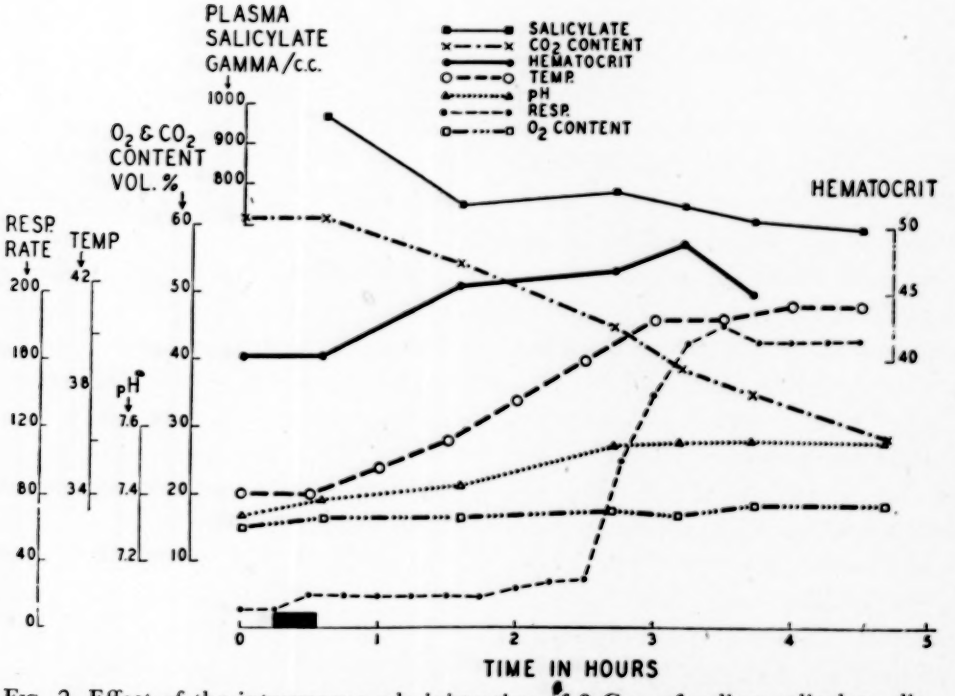


FIG. 2. Effect of the intravenous administration of 9 Gm. of sodium salicylate, dissolved in 100 cc. of an 0.85 per cent sodium chloride solution, on the acid-base balance of a dog weighing 20 Kg. under morphine-sodium barbital anesthesia.

In Figure 2 is pictured one of the nine experiments performed. In this experiment a dog weighing 20 Kg. received 1.75 cc. of a 2 per cent solution of morphine sulfate at 7:50 A.M. Between 8:50 and 9:15 A.M., the dog received intravenously 1.5 cc. per Kg. of a 20 per cent aqueous solution of sodium barbital. Respiratory frequency and temperature were followed from 9:30 A.M. on. At 11:00 A.M. an arterial blood sample was drawn.

Between 11:14 A.M. and 11:33 A.M. the dog received intravenously 9 Gm. of sodium salicylate dissolved in 100 cc. of normal saline. During the infusion no significant changes occurred in pH, $CO_2$ content, $O_2$ content or red cell volume of the arterial blood, while the temperature remained unchanged. Soon after, however, the temperature began to rise rapidly and progressively. The respiratory rate remained about the same for approximately one and one-half hours and then increased rapidly. As these changes occurred the $CO_2$ content of the arterial blood progressively decreased and the pH increased; the red cell volume increased while the oxygen content increased slightly.

## COMMENTS

It is quite clear from these experiments that the infusion of sodium salicylate *per se* does not affect the acid-base balance of the blood and that such changes as occur in the acid-base balance are produced through the action of salicylate on respiration. Indeed, the changes brought about in the $CO_2$ content and pH of the arterial blood can be adequately explained by hyperventilation. Whether sodium salicylate or its products of degradation act directly on the respiratory center or on nervous structures which in turn stimulate the respiratory center is not apparent. It seems, however, that salicylate can stimulate respiration without affecting the temperature since (1) in six of nine experiments some hyperpnea occurred before any rise in temperature, (2) in all experiments there was no parallelism between increase in temperature and increase in respiratory rate and (3) as pointed out by Guest, Rapoport and Roscoe,[3] salicylate alkalosis appears in man after hyperventilation without any rise in temperature.

## CONCLUSION

Sodium salicylate does not produce an acidosis but may produce alkalosis. The alkalosis is adequately explained by the respiratory stimulation that sodium salicylate produces. Whether hyperventilation is caused by direct or indirect stimulation of the respiration is not obvious. It is probable that the rise in temperature produced by sodium salicylate is not the only factor responsible for the respiratory stimulation.

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# Pulmonary Embolism Caused by Penicillin-Oil-Beeswax*

## *An Experimental Investigation, with Report of a Near-fatal Case*

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THE dangers of the accidental intravenous injection of drugs in oil bases are well known. Although penicillin in an oil-beeswax vehicle has been in clinical use for more than a year, up to the present no report of such a mishap with a penicillin-oil-beeswax preparation has been published. No studies have appeared of the effects of the intravenous injection of the mixture in experimental animals. This communication therefore outlines the consequences of an accidental intravenous injection of a penicillin in oil-beeswax preparation, and summarizes animal experiments in which rabbits were injected intravenously with the same material.

### CASE REPORT

L. C., an eighteen-year old colored female, was first seen in the Genitoinfectious Disease Clinic with cutaneous manifestations of secondary syphilis which were positive for Treponema pallida on darkfield examination. A serologic test for syphilis was positive in titer of 40 Kahn units.

The patient was started on a course of daily injections of 600,000 units of penicillin in oil and beeswax. Each cubic centimeter of this material contained 300,000 units of calcium penicillin suspended in peanut oil with 4.8 per cent (w/v) white wax USP. The injections were given in the upper outer quadrant of the buttock in the following manner: A sterile needle without syringe was inserted. After about fifteen seconds the syringe, which contained previously warmed penicillin-oil-beeswax preparation, was attached. As an additional precaution, the needle was aspirated and only then was the mixture injected.

Eight daily injections were given without difficulty. A few seconds after the ninth injection, however, the patient complained of a peculiar taste in her mouth, "like the smell of penicillin." She coughed a few times, but had no chest pain or dyspnea. After an hour's rest, she felt well and was sent home. The next morning she returned to the clinic. At that time, physical examination of the lungs was normal. Since the patient had no fever and felt well she was given the final injection. That afternoon, however, she began to cough again, and by evening had developed an uncomfortable shortness of breath. During the night she coughed up a considerable quantity of thick, white sputum containing yellow material which "looked like the medicine." She perspired profusely, and toward morning noticed that her sputum was blood-streaked. The shortness of breath became very severe. She returned to the clinic and was admitted to the medical ward.

At that time her temperature was 103°F., pulse 130, respirations 40, and blood pressure 130/70. She weighed 98 pounds (44.5 Kg.). The patient was a slight, well developed colored woman. She sat upright in bed and gasped for breath. She had a paroxysmal, hacking cough, productive of scanty white sputum. Her lips and fingernails were cyanotic, and there was a pronounced inspiratory nasal flare. Excursion

* From the Departments of Medicine, Pathology and Roentgenology, Emory University School of Medicine, and Grady Hospital, Atlanta, Georgia. Aided by the Bayard Sharp Fund of the Department of Pathology. Presented at the Southern Section, American Federation for Clinical Research, December 13, 1946, New Orleans, Louisiana.

FIG. 1. First hospital day, patchy densities in peripheral lung fields and reticulated pulmonary pattern; small pleural effusion, right side.

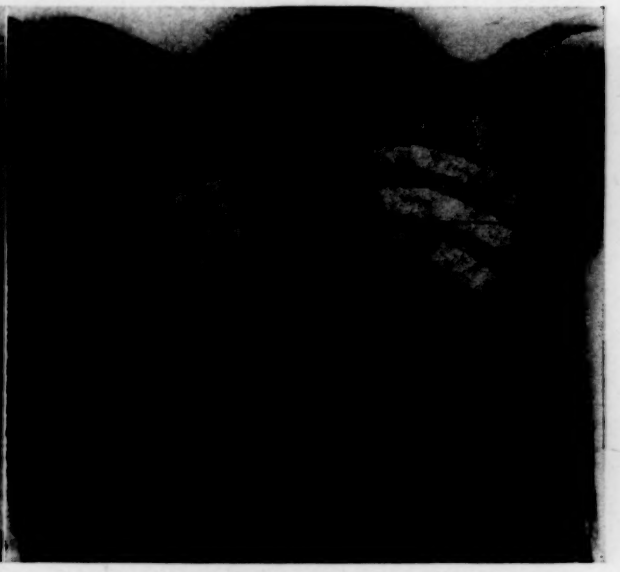

FIG. 2. Fourth hospital day, chest roentgenograms representative for period of second to fourth hospital day; increased pulmonary changes and slight cardiac enlargement; bilateral pleural effusion.

of the chest was limited symmetrically, the respirations being abdominal in character. The percussion note was resonant over the entire chest. On auscultation, a few scattered musical râles were heard posteriorly. The heart was not enlarged and no significant murmurs were heard. The second pulmonic sound was not accentuated, and neck veins were not distended.

The admission white count was 16,150 with 89 per cent polymorphonuclear leukocytes and 1 per cent eosinophiles. She was not anemic, and urine examinations were normal. No fat was found in the urine. Examination of the sputum showed a preponderance of mononuclear cells, many of which had foamy cytoplasm. Attempts to demonstrate sudanophilic fat droplets in the sputum were unsuccessful. No eosinophiles were present in the sputum. The electrocardiogram was normal. A chest roentgenogram (Fig. 1) disclosed numerous, poorly defined, patchy densities throughout both lung fields. These densities measured not more than 1 cm. in diameter and were predominantly localized in the peripheral portions of the lung fields and lung bases. The lung markings showed a streaky accentuation which resulted in a reticulated pulmonary pattern. A small amount of fluid obscured the right costophrenic angle. Fluoroscopically, the heart was normal as to size and amplitude. Both diaphragms occupied normal positions and showed satisfactory respiratory excursion.

On the first hospital day, the heart was catheterized via the left antecubital vein and the following observations made: right atrial pressure: 7 mm. Hg; right ventricular pressure: 45 mm. Hg; cardiac index (cardiac output in liters per minute per sq. meter of body surface): 2.37; arteriovenous oxygen difference: 9.1 vol. per cent. These determinations demonstrated elevation of the right ventricular pressure, with normal right atrial pressure, decreased cardiac output, and increased arteriovenous oxygen difference. These measurements are compatible with obstruction to the flow of blood through the pulmonary vascular bed. Measurements made on the eighth hospital day by the same methods showed a return to normal in all respects.

During the following days the fever gradually subsided. Dyspnea was severe on the second and third days, as reflected in respiratory rates which rose up to fifty per minute. On the second day, increasing pulmonary changes were noted by roentgenogram. The patchy densities appeared considerably larger and more numerous

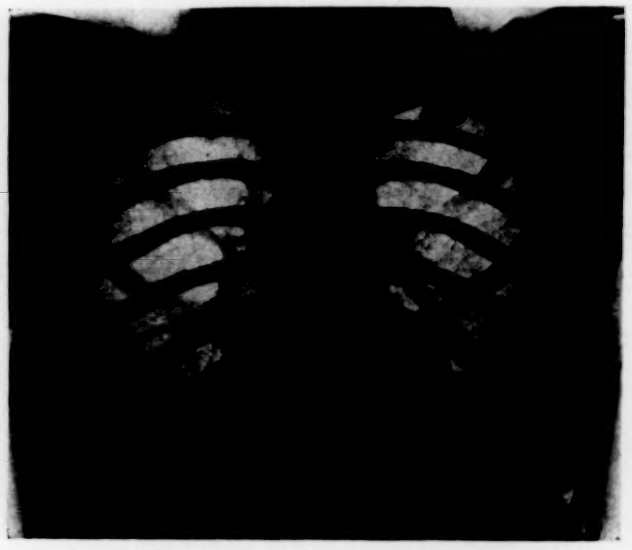

FIG. 3. Sixty-eighth day after hospital admission; no residual pulmonary or pleural changes.

and had become confluent in the axillary lung fields. Both costophrenic angles were largely obscured by pleural effusion and the cardiac silhouette had increased slightly in all diameters. The diaphragms were moderately elevated and showed diminished excursion on fluoroscopy.

On the third hospital day, the left pleural space was tapped and about 10 cc. of yellow fluid containing 33,000 cells was removed. Most of these were mononuclear cells, some being small lymphocytes and others larger monocytes and macrophage-like cells, with foamy cytoplasm. Attempts to demonstrate fat in this fluid were unsuccessful. No eosinophiles were found in the pleural fluid.

Chest roentgenograms on the fourth hospital day showed no significant change (Fig. 2); however, by the sixth day, definite evidence of resolution could be seen. At this time the white count was 9,200, of which 31 per cent were eosinophiles. The patient was discharged from the hospital in good condition six days after admission. Roentgenographic studies taken ten days after the accident showed only a few disseminated streaky densities in both lung bases. At this time the pleural effusion had completely disappeared and the heart had returned to normal size. Further examinations on the twentieth, thirty-fourth and sixty-eighth days after hospital admission revealed no abnormal roentgenologic chest findings. (Fig. 3.) The eosinophile count gradually decreased to 13 per cent forty-four days, and 5 per cent sixty-eight days after admission.

Pulmonary lipoid embolism was suspected in this patient when on admission the history was obtained that her symptoms immediately followed the ninth injection of penicillin-oil-beeswax. It was believed, however, that the patient's symptoms were unusually severe, considering that only at most 2 cc. of lipoid material could have been injected intravenously. Reports in the literature show that relatively large amounts of oil can enter a vein without producing untoward symptoms.[1,2] The following case serves to illustrate this point:

In the course of a retrograde urethrogram in a thirty-nine-year old colored man with urethral stricture, large amounts of radiopaque oil were noted in the corpora cavernosa. Ten hours later, no contrast medium remained in the corpora cavernosa, while the lung fields were diffusely mottled with the opaque material, producing a granular pulmonary pattern. At no time did the patient have respiratory symptoms.

The difference in the clinical picture of these two patients was impressive. Both had

accidentally received lipoid material intravenously. The first patient could not have received more than 2 cc. of penicillin in oil-beeswax, yet she showed a severe reaction, while the introduction of approximately 5 to 6 cc. of radiopaque oil* into the venous system of the second patient produced no symptoms whatsoever. Experiments were carried out to see if lesions could be produced which might explain the severity of the clinical picture seen in the first patient.

## EXPERIMENTAL STUDIES

*Method.* Different groups of adult rabbits were injected intravenously with 0.05 cc./Kg. of the following preparations:

1. A commercial preparation of penicillin in peanut oil-beeswax (Bristol Laboratories).
2. Peanut oil-beeswax (4.8 per cent white wax U.S.P.†) without penicillin.
3. Peanut oil containing 0.29 per cent mineral oil (w./v). This amount of mineral oil is equivalent to the amount of paraffin hydrocarbons present in 4.8 per cent white wax.
4. Peanut oil alone.
5. An aqueous solution of penicillin containing 300,000 units per cc. This is the concentration of penicillin present in the commercial preparation.

All animals survived the experiment and did not appear to be ill at its termination. Animals of each group were sacrificed by air embolism five minutes, twenty-four, forty-eight and seventy-two hours after injection. In another set of rabbits, the lethal dose of the oil-beeswax mixture was compared to the lethal dose of peanut oil alone.

Autopsy was performed immediately and the lungs removed. One lung from each animal was fixed in Zenker's fluid with 5 per cent glacial acetic acid, and the other lung in a solution of formaldehyde (10 per cent of the U.S.P. concentration). Multiple blocks representing all lobes of each lung were taken and stained with phloxine-methylene blue, Weigert's fibrin stain and with the Ziehl-Neelsen carbol fuchsin stain for the demonstration of acid-fast material. Frozen sections of comparable areas were made from the formalin-fixed tissue and stained with Herxheimer's scarlet red, Nile blue sulfate, and Fischler's method for fatty acid crystals and soaps. Blocks taken from the lungs of the animals injected with the oil-mineral oil mixture were stained for the demonstration of tissue lipase according to the method of Gomori.[3]

In certain animals, blood was drawn from the heart before injection and at the time of sacrifice for determination of blood lipase levels.[4] Lungs of animals sacrified at intervals of five minutes, twenty-four hours and forty-eight hours after injection of the test substance were analyzed chemically for total fat, neutral fat and free fatty acids.[5]

## RESULTS

*Determination of Lethal Dose.* The LD50 of peanut oil-beeswax or of penicillin-peanut oil-beeswax administered intravenously to rabbits was found to be 0.27 cc./Kg. The LD50 of peanut oil alone was 0.75 cc./Kg. (Table 1.) Calculations of LD50 were performed by the method of Reed and Muench.[6] The determination was read at the end of three days. In general, animals receiving fatal doses of peanut oil alone showed signs of cerebral involvement, such as nystagmus, convulsions and irregular slow respirations, while animals injected with lethal doses of peanut oil-beeswax died with pulmonary edema without evidence of peripheral emboli.

*Histologic Findings.* The histologic findings are identical in the seven animals injected with penicillin in peanut oil-beeswax

* Iodochloral, Searle, consists of 27 per cent iodine and 7.5 per cent chlorine in organic combination with peanut oil.

† Supplied through the courtesy of the Bristol Laboratories, Syracuse, New York.

(group 1) and in the nine rabbits injected with oil-beeswax mixture alone (group 2).

The lungs of the animals sacrificed five minutes after injection show moderately numerous small areas of recent hemorrhage and edema in the alveoli. (Fig. 4.) Many of these areas are found near arteries of varying caliber. No appreciable cellular reaction accompanies these hemorrhages. Fat stains reveal large masses of lipoid material in a number of medium-sized and large branches of the pulmonary artery. Segments of capillaries in the alveolar walls are similarly obstructed by lipoid which forms the center of the hemorrhages.

TABLE I
LETHAL DOSE ESTIMATION

| Oil-Beeswax | | | Peanut Oil Alone | | |
|---|---|---|---|---|---|
| | No. of Animals | | | No. of Animals | |
| Dose cc./Kg. | Injected | Surviving 3 Days | Dose cc./Kg. | Injected | Surviving 3 Days |
| 0.005 | 6 | 6 | 0.005 | 4 | 4 |
| 0.25 | 3 | 3 | 0.4 | 2 | 2 |
| 0.27 | 5 | 3 | 0.6 | 2 | 1 |
| 0.30 | 3 | 1 | 0.8 | 4 | 2 |
| 0.37 | 1 | 0 | 1.0 | 1 | 0 |
| 0.42 | 1 | 0 | 1.18 | 1 | 0 |

LD50 oil-beeswax: 0.270 cc./Kg.
LD50 oil alone: 0.747 cc./Kg.

In animals sacrificed twenty-four hours after injection, hemorrhages are still present. These are now attended by a fair degree of cellular infiltration consisting of numerous heterophiles and some mononuclear cells and lymphocytes. The periphery of the lesions shows edema and congestion. The lesions are moderately numerous and are found scattered throughout the sections. Some medium-sized and large arteries show recent thrombi which have elicited endothelial proliferation. The adventitia of the vessels displays marked cellular infiltration which is similar to that encountered in the areas of hemorrhage. This infiltration often extends into the adjacent alveoli. In some instances, early granulomatous foci are seen. In the hemorrhagic areas fat stains reveal lipoid masses within the capillaries of the alveolar septa which are undergoing necrosis. The same lipoid material is also found in the thrombi of the larger vessels. Large mononuclear cells begin to invade the lipoid and often show small phagocytized fat globules. At this stage fibroblastic proliferation is inconspicuous around the hemorrhages or thrombosed blood vessels.

Granulomatous lesions predominate in the lungs of animals sacrificed forty-eight hours after injection. Most of the lesions are ill-defined but some are already fairly well demarcated. These granulomas form in the areas of hemorrhage and are also found around the thrombosed vessels where they extend into the adventitia. The core of the granulomas consists of one or several masses of lipoid material surrounded by a few giant cells, large mononuclear cells and lymphocytes, as well as quite numerous heterophiles and occasional eosinophiles. At this stage, early proliferation of fibroblasts is noted. The lipoid material in the capillaries and larger vessels is undergoing further fragmentation and phagocytosis. The thrombi in the larger vessels display more advanced endothelial proliferation and also beginning organization. Occasional areas of hemorrhage are still present throughout the parenchyma.

A similar picture is seen in the lungs of animals sacrificed seventy-two hours after injection. Here the organization of the thrombi in the large blood vessels is quite advanced. (Fig. 5.) The granulomas are now well defined, contain prominent large multinucleated giant cells and consist chiefly of large mononuclear cells, some lymphocytes, occasional eosinophiles and rare heterophiles. (Fig. 6.) Distinct fibroblastic

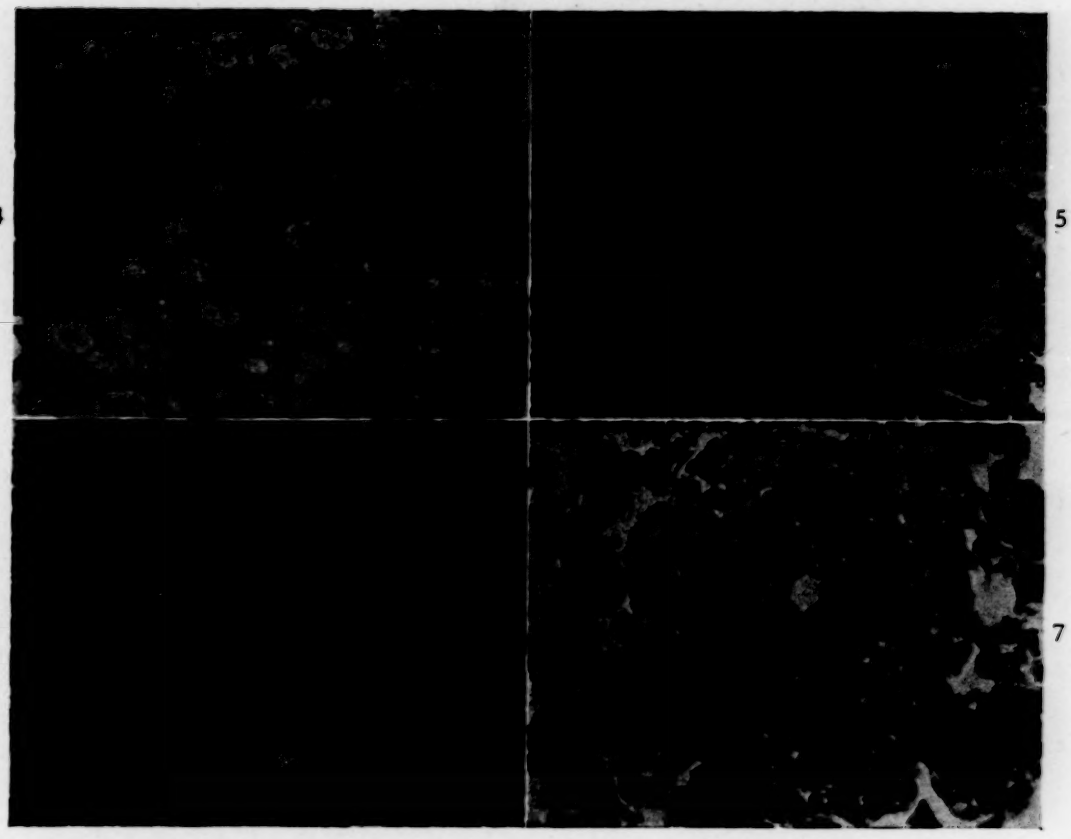


FIG. 4. Rabbit injected with peanut oil-beeswax and sacrificed after five minutes. There is marked patchy edema with slight recent hemorrhage; phloxine-methylene blue. × 80.
FIG. 5. Rabbit injected with peanut oil-beeswax and sacrificed after three days. A large branch of the pulmonary artery is obstructed by a thrombus undergoing organization; phloxine-methylene blue. × 120.
FIG. 6. Rabbit injected with penicillin in peanut oil-beeswax and sacrificed after three days. There are several large and confluent granulomas surrounded by marked cellular reaction (compare with Figs. 8 and 10); phloxine-methylene blue. × 80.
FIG. 7. Granuloma produced by penicillin in peanut oil-beeswax after three days. The space in the center is a mass of lipoid surrounded by a marked chronic inflammatory cell reaction which includes proliferating fibroblasts (compare with Figs. 9 and 11); phloxine-methylene blue. × 330.

proliferation is seen at the periphery of the lesion. (Fig. 7.)

The findings in the lungs of the four animals injected with the peanut oil—mineral oil mixture (group 3) resemble those which have already been described. The granulomas are similar in distribution, size, and cellular components and also show distinct fibrosis. (Figs. 8 and 9.) There is, however, a difference in the size of the arteries which are thrombosed. The large branches of the pulmonary artery are not involved and thrombi are found only in vessels of smaller caliber.

Nine rabbits were injected with peanut oil alone (group 4). The lungs of the animals sacrificed five minutes after injection show nothing of note with ordinary stains. Fat stains reveal lipoid masses in the capillaries of many alveoli but only a few small arteries are involved.

After twenty-four hours, a fair number of small early granulomas are seen in the alveolar septa and around the few larger

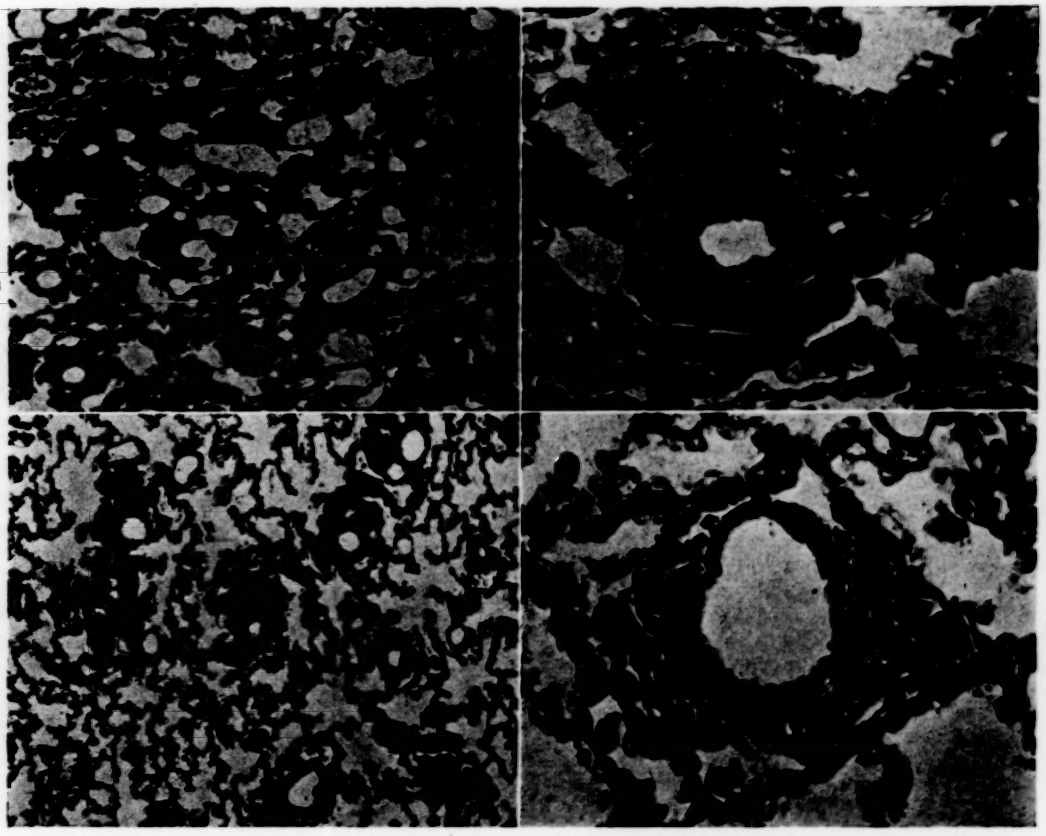

FIG. 8. Rabbit injected with peanut oil-mineral oil and sacrificed after three days. These granulomas are small but tend to be confluent (compare with Figs. 6 and 10); phloxine-methylene blue. × 80.

FIG. 9. Granuloma produced by peanut oil-mineral oil after three days. The central space is a mass of lipoid surrounded by a reaction similar in intensity and cellular components to the reaction produced by penicillin in peanut oil-beeswax (compare with Figs. 7 and 11); phloxine-methylene blue. × 330.

FIG. 10. Rabbit injected with peanut oil alone and sacrificed after three days. The granulomas, like those produced by peanut oil-mineral oil, are small but show less reaction (compare with Figs. 6 and 8); phloxine-methylene blue. × 80.

FIG. 11. Granuloma produced by peanut oil alone after three days. The large central space is a mass of lipoid surrounded by a comparatively slight cellular reaction without appreciable proliferation of fibroblasts (compare with Figs. 7 and 9); phloxine-methylene blue. × 330.

vessels which are involved. The cellular infiltration consists chiefly of heterophiles and some large mononuclear cells and lymphocytes. This infiltration involves the adjacent alveoli. Fat stains show fragmentation and phagocytosis of the lipoid material.

After forty-eight hours the granulomas are well defined and consist mainly of large mononuclear cells and lymphocytes, as well as some multinucleated giant cells. Even after seventy-two hours the granulomas in these animals are smaller than those in the other series. (Fig. 10.) A slight degree of fibroblastic proliferation is seen. The degree of connective tissue response is less marked in granulomas produced by peanut oil than in lesions of similar size and location produced by the other lipoid substances and after seventy-two hours the fibrosis is not appreciably more marked. (Fig. 11.) Only a few vessels larger than capillaries are involved and those are small arteries.

The various fat stains were positive in

demonstrating the presence of lipoid but were entirely inconclusive as to the proportion of free fatty acids.

The lungs of the animals injected with an aqueous solution of penicillin (group 5) and sacrificed after five minutes and three days showed nothing remarkable.

*Chemical Analyses.* The histochemical demonstration of lipase in the lungs of the animals injected with peanut oil-mineral oil (group 3) shows small amounts of enzyme in the lesions of twenty-four hours' duration. After forty-eight hours, large amounts of lipase are found while after seventy-two hours the amount of enzyme in the lesions is markedly decreased. The enzyme is seen only in the large mononuclear cells and giant cells of the lesions. In all sections the bronchial epithelium showed the normal positive reaction for the enzyme.

Assays of lungs for fat and fatty acids failed to reveal any increase of the fatty acid content of rabbit lungs twenty-four and forty-eight hours after injection of oil or oil-beeswax. No demonstrable change occurred in the concentration of blood lipase during the course of the experiments.

## COMMENTS

The difference in the viscosity of penicillin in peanut oil-beeswax and peanut oil alone is obvious. The oil-beeswax mixture is solid at room temperature and will barely flow at body temperature. Peanut oil is fluid at room temperature. The addition of the small amount of mineral oil involved in these experiments does not appreciably alter its viscosity. The difference in fluidity is reflected in the results of the animal experiments. Sections of the lungs show that the peanut oil and peanut oil-mineral oil pass through the larger vessels and are trapped by the capillaries; on the other hand, most of the oil-wax mixture is arrested in the larger branches of the pulmonary artery. Penicillin does not appear to influence the location of the emboli. The tendency of the viscous oil-beeswax mixture to be trapped by the larger arteries may in part account for the severity of symptoms in the first case. The quantity of oil-beeswax injected was small; but since it was arrested in the larger vessels, it obstructed the blood supply to a relatively large portion of the lung. In the second case, the oil was dispersed into the pulmonary capillary bed. Since the total diameter of the pulmonary capillary bed is far greater than that of the large arteries, the oil obstructed the blood flow to a relatively insignificant amount of pulmonary tissue, and no symptoms resulted.

The difference in reaction to peanut oil-beeswax and to peanut oil alone does not appear to be confined to purely mechanical factors. The cellular reaction to the oil-beeswax mixture appears to be more intense than that to peanut oil alone, and is associated with an appreciable amount of fibrous tissue proliferation. A comparison of lesions of similar size and location shows that the connective tissue reaction is inconspicuous in the lesions produced by peanut oil alone. This difference suggests a chemical factor. The greater degree of reaction in the lesions produced by the peanut oil-beeswax mixture may be related to the chemical composition of the beeswax. White wax, USP, is a mixture of many substances, which include polyhydric alcohols, long-chain fatty acids and cyclic compounds. It also contains approximately 6 per cent paraffin hydrocarbons.[7] The addition of paraffin hydrocarbon, in the form of mineral oil, to peanut oil, produced granulomas quite similar in cytology to those caused by the oil-beeswax mixture but involving small arterioles and capillaries. This finding suggests that the difference in the tissue response between the granulomas produced by peanut oil and by peanut oil-beeswax may be due to the small amounts of paraffin hydrocarbon present in the beeswax. The

histologic pattern of the granuloma produced by oil-beeswax resembles that found in lipoid pneumonia.

The comparison of the LD50 of peanut oil-beeswax and oil alone emphasizes the relative toxicity of the two substances. It was found that the preparation containing beeswax was 2.8 times as toxic as peanut oil alone.

Several observers have remarked upon the latent period which may be found between the occurrence of pulmonary fat embolism and the development of severe symptoms.[8,9] Our patient was relatively asymptomatic immediately after the offending injection. After twenty-four hours, however, she began to develop pulmonary distress which became progressively more severe until it reached a peak on the second hospital day (fourth day of present illness). Harris and co-workers[10] have postulated that the late appearance of symptoms is the result of hydrolysis of the fat particles with release of free fatty acids. It is known that the severity of the tissue reaction to lipoids increases with their free fatty acid content. An appreciable increase in lipase activity was found in the granulomas produced by the oil-mineral oil mixture after forty-eight hours. The delay in the development of maximal lipase activity correlates roughly with the appearance of the granulomas in the experimental animals. In an analogous fashion, the roentgenographic findings in our patient indicated a progression of the pulmonary involvement, reaching its maximum between the second and fourth hospital days.

A comparison of clinical and roentgenographic findings with the experimental data might be interpreted as offering support for the concept that the increase of the patient's symptoms is related to the enzymatic release of irritating free fatty acids from the relatively bland neutral fat. Other histologic technics, as well as chemical analyses were inconclusive, possibly because the methods at our disposal were not sufficiently sensitive.

The first patient developed a marked eosinophilia during her stay in the hospital. There were no cutaneous manifestations of allergy, and no ova of intestinal parasites were found. No eosinophiles were found in the sputum or pleural exudate. Subsequent investigation of a large number of patients receiving penicillin in oil-beeswax intramuscularly in the Genitoinfectious Disease Clinic revealed that approximately 10 per cent of all patients receiving the preparation have elevated eosinophile counts, ranging from 10 to 30 per cent.[11] We believe that the eosinophilia in our patient is not indicative of the presence of allergic disease. The coincidence of pulmonary disease and eosinophilia raises the question of Loeffler's syndrome. The roentgenographic findings, severity of the disease, and spontaneous rapid resolution in a period of less than two weeks are incompatible with this diagnosis.

The roentgenographic findings are of interest because the symmetrical, peripheral distribution of the opacities is similar to that seen in protracted types of hematogenous tuberculosis and in diffuse pulmonary carcinomatosis. This similarity suggests additional evidence that our patient's symptoms arose following multiple pulmonary emboli.

These studies indicate that every precaution should be taken to prevent the intravenous administration of oil-beeswax preparations. The danger involved is greater than when oil alone is involved. If an oil-beeswax mixture is injected intravenously, the patient should be kept under close observation. For twenty-four hours after the accident, our first patient gave no indication of the severity of the impending reaction. At the time of admission, thirty-six hours after the injection, roentgenograms of the chest showed extensive involvement of the lung. Clinically and roentgenologically the pulmonary findings were most pronounced

on the second and third hospital days. There was a rapid clinical improvement thereafter, though the roentgenograms showed a somewhat delayed resolution of the pathologic process. It is noteworthy that roentgenologic and clinical examinations twenty days after the injection showed no residual pulmonary changes. It therefore seems advisable to hospitalize patients suspected of having had an oil-beeswax mixture injected intravenously. Such patients should be observed for at least three days before the possibility of a severe late reaction is discarded.

## CONCLUSIONS

A case is reported of a severe reaction to the accidental intravenous administration of penicillin in oil-beeswax. Animal experiments were done which indicated that the severity of the reaction was due to two factors: first, the viscosity of the oil-beeswax mixture which caused it to block large branches of the pulmonary artery, and second, the severe inflammatory reaction elicited by the beeswax.

Evidence was obtained which suggested that the severity of the inflammation may be due to the chemical composition of the wax. Attempts to correlate the morphology of the lesions in experimental animals with the chemical demonstration of free fatty acids in tissues were inconclusive.

The importance of precaution against intravenous injection of penicillin-oil-beeswax is stressed.

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# The Electrocardiogram in Lupus Erythematosus Disseminatus*

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THE involvement of the heart in disseminated lupus erythematosus is well known. There may be vegetations of the endocardium, first described by Libman and Sacks.[1] The myocardium is often the seat of focal inflammation and the pericardium may become thickened and adherent with resulting synechiae cordis or even complete obliteration of the pericardial sac. Laipply and Longley[2] are of the opinion that the pathologic picture is not definite but Klemperer, Pollack and Baehr[3] believe that the changes, wherever they occur in the body, are due to collagenous proliferation and degeneration.

It is to be expected that such marked anatomic changes in the heart would have an effect on the electrocardiogram. The present study was made to review the literature on this subject and to add the observations in eight additional cases of disseminated lupus erythematosus proved by post mortem examination. The authors have been unable to find any articles dealing with the electrocardiographic changes alone but there are numerous references in the literature to the changes in the electrocardiogram in case reports of disseminated lupus. Baehr,[4] in his account of the disease in Cecil's Textbook of Medicine, states: "The electrocardiogram reveals no characteristic changes as a rule except for low voltage." In a brief review of the literature of lupus erythematosus disseminatus Cluxton and Krause[5] make the same statement. In their case reports an electrocardiogram taken on each of two (out of four) cases was normal in one instance and in the other showed "notching of T in Lead IV." Isolated case reports[6] have noted low or inverted T waves in Leads I, II and IV, increased P-R interval and increase in left axis deviation. Bunim[7] reported low voltage and premature ventricular and "His bundle" contractions; in another case report the Reifensteins[8] also noted low voltage. In both of these instances postmortem examination revealed chronic pericarditis with fibrous adhesions between visceral and parietal pericardium. This was the only cardiac abnormality in one case; the other had, in addition, vegetative endocarditis. Baehr, Klemperer and Schifrin[9] found that "the only abnormality characteristic of all electrocardiograms was low voltage" but do not state whether or not electrocardiograms were taken in all instances.

In this report the electrocardiograms of eight cases of lupus erythematosus disseminatus were analyzed.

## FINDINGS

1. The electrocardiograms of four patients were normal. In one case the record was made three years and nine months before death but in the other three instances the records were made fifty-five, thirty and thirteen days before death. At postmortem examination one heart was normal; one

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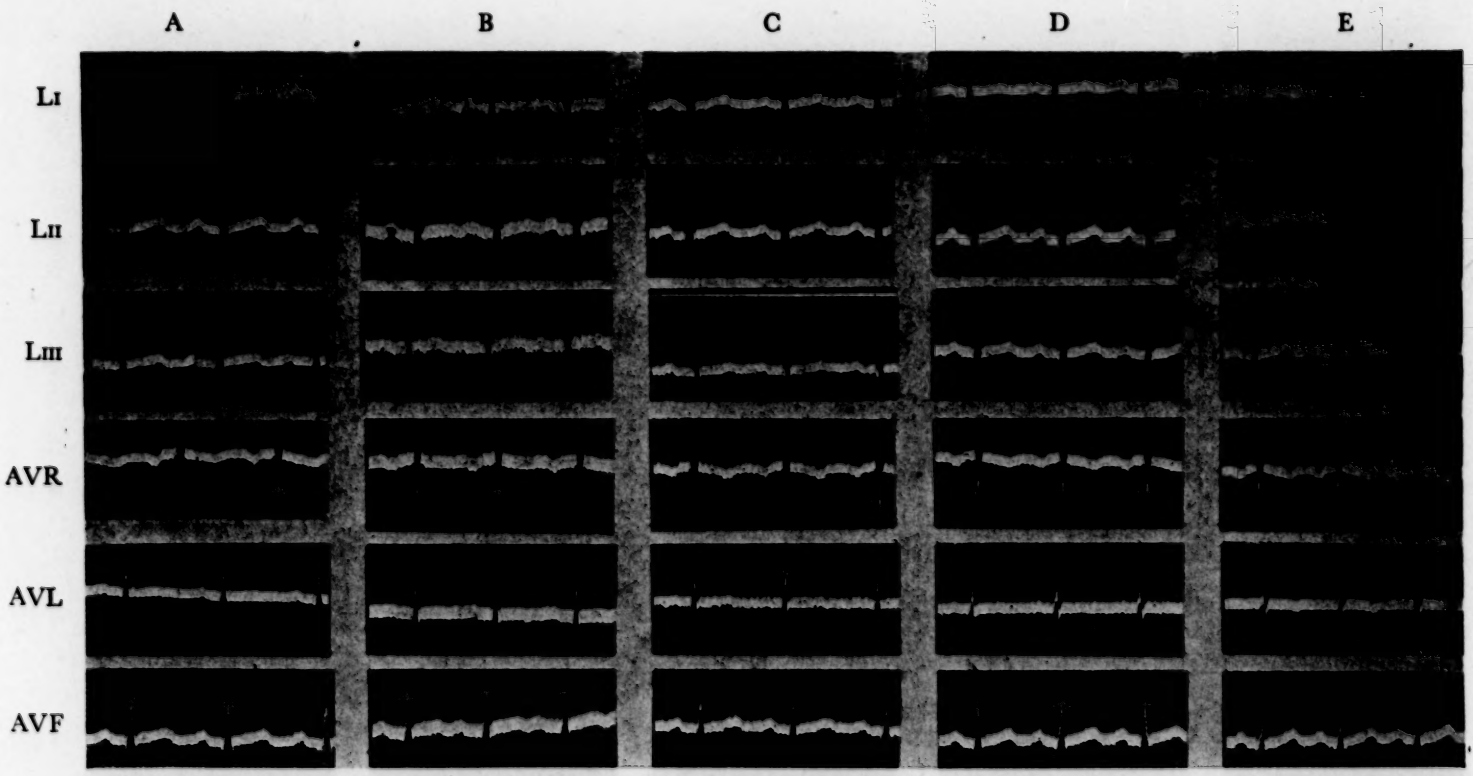
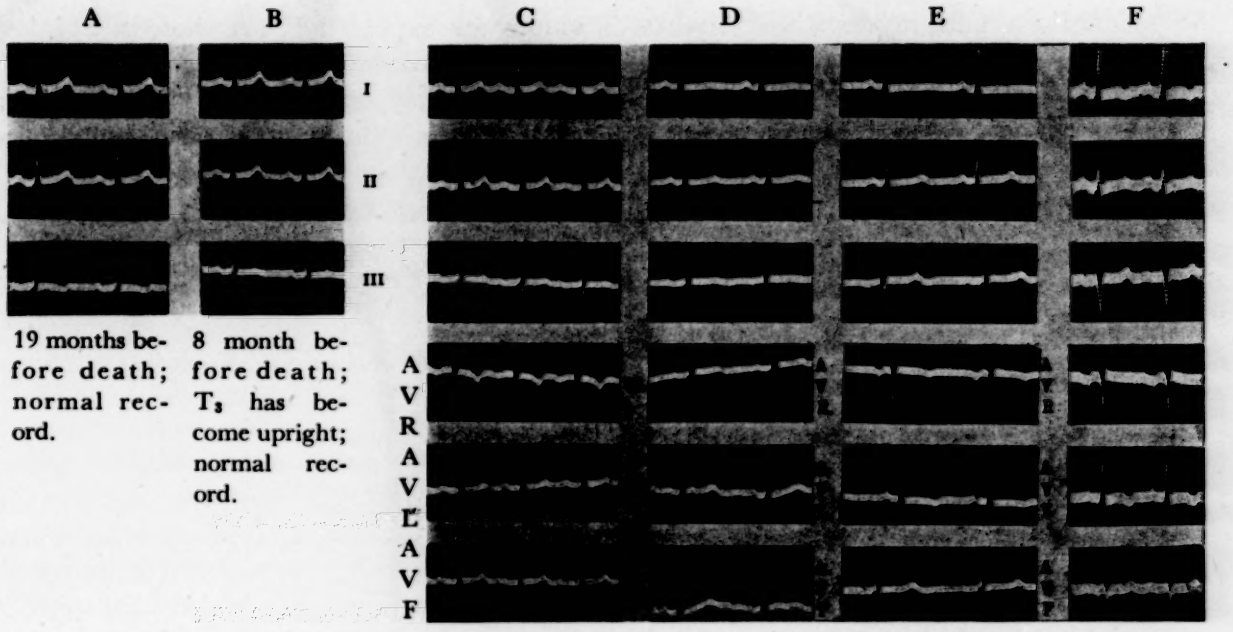



| A | B | C | D | E |
|---|---|---|---|---|
| 96 days before death. P-R (.21 sec.) prolonged for rate (92). | 84 days before death. P-R (.20 sec.) prolonged for rate (112). | 50 days before death. P-R (.20 sec.) prolonged for rate (98). | 26 days before death. P-R now normal. $T_1$ has become smaller and $T_{AVL}$ isolectric. | 5 days before death. Voltage in limb leads now abnormally small. $T_1$ is now almost isoelectric and $T_{AVL}$ now inverted. |

FIG. 1. O. N., female; anatomical diagnoses (cardiac); healed verrucose endocarditis of mitral and tricuspid valves.

FIG. 2. T. P., male; anatomical diagnoses (cardiac); acute vegetative endocarditis of all four valves; subacute myocarditis, hydropericardium (450 cc.), hypertrophy and dilatation of the heart (415 Gm.).




19 months before death; normal record.

8 month before death; $T_3$ has become upright; normal record.

7 months before death; $T_1$ and $T_2$ have decreased in amplitude.

54 days before death; $T_1$ now abnormally small; $T_2$ further reduced; amplitude of inverted $QRS_3$ has increased (increase in l.a.d.).

25 days before death; $T_1$ now almost isoelectric; $T_{AVL}$ now inverted; amplitude of $QRS_3$ has increased further.

9 days before death; $T_1$ now inverted; Q-T abnormally prolonged; augmented leads also show increase in l.a.d.

FIG. 3. C. L., female; anatomical diagnoses (cardiac); miliary abscesses of epicardium and myocardium, hydropericardium (300 cc.), cardiac hypertrophy (380 Gm., expected weight 290 Gm.).

had focal myocarditis, endocarditis and mitral valvulitis; one had synechiae cordis and mitral valvulitis; one had synechiae cordis, myocarditis and mitral and aortic valvulitis. (Table II.)

2. In two patients the electrocardiograms showed abnormally low voltage (amplitude of QRS less than 5 mm. in all limb leads). One patient had complete obliteration of the pericardial space by fibrous adhesions, plus mitral and pulmonary valvulitis. Low voltage was evident ten months before death. The other had healed verrucous endocarditis of the mitral and tricuspid valves; the pericardium, myocardium and the lining endocardium were normal. In this patient low voltage was first found five days before death.

3. Progressive T wave changes occurred in the electrocardiograms of three patients. (Figs. 1, 2, and 3.) The first (Fig. 1) had healed mitral and tricuspid valvulitis; the lining endocardium, myocardium and pericardium were normal. The second (Fig. 2) had acute vegetative endocarditis of all four valves, subacute myocarditis, hydropericardium (450 cc.) and cardiac hypertrophy and dilatation (415 Gm.). The third (Fig. 3) showed miliary abscesses of

the epicardium and myocardium, hydropericardium (300 cc.) and cardiac hypertrophy (380 Gm.).

4. Axis change was seen in two patients. In one the axis shifted from 90° to abnormal right axis deviation. In the other a slight left axis deviation became more marked. (Figs. 2 and 3.)

5. P-R was prolonged in one patient. (Fig. 1.)

6. The Q-T interval was prolonged beyond the upper limit of normal in two patients. In three other patients Q-T fell within the normal range but was longer than average. This marked tendency toward prolongation of Q-T, definitely abnormal in two patients, can be readily seen in Table I. In this table "K" was calculated from the formula $K = \frac{Q\text{-}T}{\sqrt{R\text{-}R}}$. The "Normal" and "Upper Limit of Normal" values for expected K are those of Shipley and Halloran.[10]

TABLE I

| Case | Record | R-R | Q-T | K | Expected K | |
|---|---|---|---|---|---|---|
| | | | | | Normal | Upper Limit of Normal |
| O. N. ♀ | 1 | .66 | .35 | .432 | .415 | .456 |
| | 2 | .54 | .32 | .436 | .415 | .456 |
| | 3 | .62 | .34 | .432 | .415 | .456 |
| | 4 | .56 | .31 | .415 | .415 | .456 |
| | 5 | .52 | .30 | .416 | .415 | .456 |
| M. R. ♀ | | .58 | .30 | .394 | .415 | .456 |
| V. C. ♀ | | .72 | .33 | .389 | .415 | .456 |
| S. K. ♀ | | .60 | .35 | .452 | .415 | .456 |
| E. B. ♀ | | .80 | .39 | .436 | .415 | .456 |
| T. P. ♂ | 1 | .91 | .41 | .419 | .397 | .433 |
| | 2 | .68 | .38 | .461 | .397 | .433 |
| | 3 | .56 | .35 | .468 | .397 | .433 |
| M. S. ♀ | | .53 | .28 | .385 | .415 | .456 |
| C. L. ♀ | 1 | .59 | .32 | .417 | .415 | .456 |
| | 2 | .69 | .36 | .434 | .415 | .456 |
| | 3 | .52 | .29 | .402 | .415 | .456 |
| | 4 | .69 | .36 | .434 | .415 | .456 |
| | 5 | .83 | .40 | .440 | .415 | .456 |
| | 6 | .56 | .36 | .482 | .415 | .456 |

A summary of the electrocardiographic and anatomic findings is seen below, in Table II.

TABLE II

| Case | Pathologic Findings | Electrocardiographic Findings |
|---|---|---|
| S. K. ♀ | Chronic pericarditis with obliteration of pericardial space by fibrous adhesions<br>Chronic mitral and pulmonary valvulitis (undetermined type) | Low voltage |
| M. R. ♀ | Focal myocarditis and necrosis of arterioles<br>Acute mural endocarditis<br>Acute mitral valvulitis | Normal (taken 13 days before death) |
| V. C. ♀ | Normal heart | Normal (taken 55 days before death) |
| M. S. ♀ | Synechiae cordis with complete obliteration of pericardial space.<br>Mild cardiac hypertrophy (500 Gm.)<br>Healed mitral valvulitis | Normal (taken 30 days before death) |
| E. B. ♀ | Synechiae cordis<br>Acute and subacute myocarditis<br>Chronic proliferative and verrucose mitral and aortic valvulitis | Normal (taken 3 years and 9 months before death) |
| O. N. ♀ | Healed verrucose endocarditis of mitral and tricuspid valves | Prolonged P-R interval<br>Low voltage<br>Flattening of $T_1$; inversion of $T_{IVL}$ |
| T. P. ♂ | Acute vegetative endocarditis of all four valves<br>Subacute myocarditis<br>Hydropericardium (450 cc.)<br>Hypertrophy and dilatation of heart (415 Gm.) | Q-T abnormally prolonged<br>Change to right axis deviation<br>Inversion of $T_1$; flattening and diphasicity of $T_2$ and $T_{IVR}$; flattening of $T_3$ |
| C.L. ♀ * | Miliary abscesses of epicardium and myocardium<br>Hydropericardium (300 cc.)<br>Cardiac hypertrophy (380 Gm.)<br>Chronic epicarditis | Q-T abnormally prolonged<br>Increase in left axis deviation<br>Inversion of $T_1$; decrease in amplitude of $T_2$; change of $T_3$ from inverted to upright |

* This patient's illness was complicated by terminal pyemia (alpha hemolytic streptococcus and hemolytic staphylococcus).

SUMMARY

Abnormalities of the electrocardiogram in patients with lupus erythematosus disseminatus have been noted by several authors in case reports. These abnormalities have consisted of low voltage, increased P-R interval, increase in left axis deviation, low or inverted T waves and premature beats.

This report presents the electrocardiographic findings in eight autopsied cases of lupus erythematosus disseminatus. All abnormalities noted above were found in this group with the exception of premature beats. In addition, change of axis from normal to right axis deviation occurred. There was also a general tendency toward prolongation of the Q-T interval; in two patients Q-T was abnormally prolonged. Low voltage was not as common as reported in other series.

Electrocardiographic changes were seen as long as ten months before death. In those patients in whom a series of records was obtained the electrocardiograms became progressively and increasingly abnormal. In three of eight patients in whom there was anatomic abnormality of the heart there was no change in the electrocardiogram. In all of these, however, there was no series of records; only a single electrocardiogram was available for study.

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# Metopon Hydrochloride

## *Methyldihydromorphinone Hydrochloride*

STATEMENT OF COMMITTEE ON DRUG ADDICTION, NATIONAL RESEARCH COUNCIL

In 1929 with the funds provided by the Rockefeller Foundation, the National Research Council through its Committee on Drug Addiction undertook a coordinated program to study drug addiction and search for a non-addicting analgesic comparable to morphine. The principal participating organizations were the Universities of Virginia and Michigan, the United States Public Health Service, the Treasury Department's Bureau of Narcotics and the Health Department of the State of Massachusetts, which brought together chemical, pharmacological and clinical facilities for the purpose of the study. Metopon is one of the many compounds made and studied in this coordinated effort.

Chemically, metopon is a morphine derivative; pharmacologically it is qualitatively like morphine even to the properties of tolerance and addiction liability. Chemically, metopon differs from morphine in three particulars—one double bond of the phenanthrene nucleus has been reduced by hydrogenation, the alcoholic hydroxyl has been replaced by oxygen and a new substituent, a methyl group, has been attached to the phenanthrene nucleus. Studies made thus far indicate that pharmacologically metopon differs from morphine quantitatively in all of its important actions. Its analgesic effectiveness is at least double and its duration of action is about equal to that of morphine; it is nearly devoid of emetic action; tolerance to it appears to develop more slowly and disappears more quickly and physical dependence builds up more slowly than it does with morphine; therapeutic analgesic doses produce little or no respiratory depression and much less mental dullness than does morphine and it is relatively highly effective by oral administration.

In addition to animal experiments these differences have been established by extensive employment of the drug in two types of patients, individuals addicted to morphine and others (terminal malignancies) needing prolonged pain relief but without previous opiate experience. In morphine addicts metopon appears only partially to prevent the impending signs of physical and psychical dependence. In terminal malignancy, administered orally, it gives adequate pain relief with very little mental dulling, without nausea or vomiting and with slow development of tolerance and dependence.

The high analgesic effectiveness of oral doses (with the elimination of the disadvantage to the patient of hypodermic injection), the absence of nausea and vomiting even in patients who vomit with morphine or other derivatives, the absence of mental dullness and the slow development of tolerance and dependence place metopon in a class by itself for the treatment of the chronic suffering of malignancies and it is for that purpose exclusively that it is being manufactured and marketed.

Metopon will be available only in capsule form for oral administration. The capsules will be put up in bottles of one hundred and each capsule will contain 3.0 mg. of metopon hydrochloride. They can be obtained by physicians from only

Sharp & Dohme or Parke, Davis & Co., on a regular official Narcotic Order Form which must be accompanied by a signed statement supplying information as to the number of patients to be treated and the diagnosis on each. The drug will be distributed for no other purpose than oral administration for chronic pain relief in cancer cases.

The dose of metopon hydrochloride is 6.0 to 9.0 mg. (2 or 3 capsules) to be repeated only on recurrence of pain, avoiding regular by-the-clock administration. As with morphine it is most desirable to keep the dose at the lowest level compatible with adequate pain relief, therefore administration should be started with 2 capsules per dose increasing to 3 only if the analgesic effect is insufficient.

Tolerance to any narcotic drug develops more rapidly with excessive dosage and under regular by-the-clock administration. Also as a rule the pain of cancer varies widely in intensity from time to time. Pain, therefore, should be the only guide to time of administration and dosage level. Tolerance to metopon hydrochloride develops slowly. It can be delayed or interrupted entirely by withholding the drug occasionally for twelve hours or for as much of that period as the incidence of pain will permit.

A record card will be sent to each physician for each patient to whom metopon hydrochloride is to be administered. He will be requested to fill out these cards and return them in the addressed return envelope. He must furnish this record of the patient and his use of metopon hydrochloride if he wishes to repeat his order of the drug. The principal object of this detailed report is to check the satisfactory results of metopon hydrochloride administration in general practice. The physician's cooperation in making it as complete as possible is earnestly solicited.

The limited use of metopon hydrochloride as described above has been recommended by the Drug Addiction Committee of the National Research Council, and the Committee, with the cooperation of the American Cancer Society, will supervise the distribution of the drug. The Committee is composed of William Charles White, Chairman, Washington, D. C.; H. J. Anslinger, Commissioner of Narcotics, United States Treasury Department, Washington, D. C.; Lyndon F. Small, National Institute of Health, Washington, D. C. and Nathan B. Eddy, National Institute of Health, Washington, D. C. Queries and comments on metopon may be directed to Dr. Eddy who will answer them on behalf of the Committee.

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# Review

# Clinical Syndromes Associated with Gonadal Failure in Men*

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THE testes have two functioning systems, one composed of the interstitial or Leydig cells with internal secretory activity for the elaboration of male sex hormone, testosterone;[1,2] the other made up of the seminiferous tubules for the production of mature spermatozoa. Although some internal secretory activity begins shortly after birth,[3] no apparent spermatogenic function is noted until puberty. At that time, secondary to spontaneous hypophyseal gonadotropic activity,[4] both testicular systems mature and become active, usually for the remainder of the individual's span.[5] Either or both testicular functions may fail to achieve normal activity at puberty, or to maintain this level once function has been established. The clinical picture which ensues then depends on the function lost and on the time in relation to maturation.

The establishment and maintenance of adult testicular function depends upon normal genetic, embryologic and physiologic development and upon avoidance of disease or injury to the organ. Any disturbance in the above influences may derange testis function. Such disorders are herein outlined to provide a working etiologic basis of classification for purposes of discussion.

### DISORDERS OF THE TESTES

I. Prepuberal, Prior to Sexual Maturation
  A. Primary, or in the testes proper
    1. Non-destructive
      (a) Genetic: defective germ plasm anlage
        (1) Eunuchoidism—some cases.
        (2) Aplasia testis (?)
      (b) Physiologic: hormonal excesses affecting the fetus in utero —pseudohermaphroditism
    2. Destructive
      (a) Embryologic: disturbances in descent of the testes
        (1) Compromise of blood supply with atrophy, aplasia testis (?)
        (2) Cryptorchidism
          (*a*) Complete tubule destruction by body heat at puberty
          (*b*) Incomplete interstitial cell function loss
        (3) Maldescent of the testes; no function loss other than with cryptorchidism
      (b) Pathologic: mumps, malignancy, operation, injury, etc. (See II,A.2.)
  B. Secondary, or elsewhere than in the testes
    1. Disturbance of the anterior hypophysis proper
      (a) Non-destructive
        (1) Normal onset of gonadotropic activity as late as sixteen years of age

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(2) Seasonal inactivity—animals only
(3) Eunuchoidism—some cases
(4) Dwarfism (genetic, somatic, vs. hypophyseal origin of this disorder)

(b) Destructive
(1) Adenomas of the anterior hypophysis—usually postpuberal
(2) Rathke's pouch cyst or tumor
(3) Fröhlich's syndrome
(4) Carcinoma, tuberculosis and miscellaneous diseases of the hypophysis

2. Disease elsewhere than the hypophysis; interrelationship with anterior hypophyseal function
(a) Diabetes mellitus
(b) Starvation and inanition
(c) Vitamin deficiency, especially thiamin, vitamin A
(d) Defective absorption from gastrointestinal tract, as in chronic diarrhea, sprue
(e) Chronic renal disease
(f) Thyroid disorders
(1) Hypothyroidism
(2) Hyperthyroidism
(g) Adrenal disorders
(1) Addison's disease
(2) Cushing's syndrome
(3) Cortical hyperplasia and adenoma

II. Postpuberal, after Sexual Maturation
A. Primary, or in the testes proper
1. Non-destructive
(a) Male climacterium
(b) Sterility—genetic?
2. Destructive
(a) Orchitis—usually unilateral
(1) Mumps
(2) Tuberculosis
(3) Lues
(b) Malignancy—usually unilateral[6]
(1) Origin in embryonal rest cells
(*a*) Chorionepithelioma
(*b*) Teratoma
(2) Origin in seminiferous epithelium—seminoma
(3) Origin in interstitial tissue
(*a*) Adenoma
(*b*) Hyperplasia
(4) Origin in connective tissue
(*a*) Fibroma
(*b*) Myoma
(c) Compromise of blood supply, as after hernia repair
(d) Castration—carcinoma of prostate, etc.
(e) Injury—avulsion, etc.
(f) *X*-radiation
(g) Chemicals—e.g., alcohol, toxins
(h) Abnormally high body temperatures

B. Secondary, or elsewhere than in the testes
1. Disturbance of the anterior hypophysis proper
(a) Non-destructive—? actually occurs
(b) Destructive
(1) See outline I, B.
(2) Aneurysm internal carotid artery
2. Disease elsewhere than the hypophysis; interrelationship with anterior hypophyseal function
(a) See outline I, B, 2.
(b) Estrogen or androgen excess
(1) Therapeutic administration
(2) Failure of inactivation of endogenous hormone, in liver disease

## GONADAL DEVELOPMENT AND DIFFERENTIATION

The testes arise from the genital ridge on either side of the human embryo at the 20 mm. stage, in the seventh week of pregnancy. The cells begin to differentiate into tubule and interstitial cells at this time, although this process may be renewed after birth. Migration of the testes to the scrotum commences at the 41 mm. stage in the third month and descent is completed one to two months after birth.

The differentiation of the gonad into a male or female organ is under genetic or chromosomal control but may be altered by unusual hormonal influence during gestation.[7] Such hormonal excess may come from the mother, a twin embryo (in the cow), or from the endocrine system of the fetus itself. In the last instance, the fetal overactivity may have been initiated by either of the other two. Pseudohermaphroditism in man is an example of this. The overactivity of the fetal adrenal cortex occasionally continues after birth, as shown by enlarged adrenal cortices[8] and by an excess excretion of neutral 17-ketosteroids in the urine of the human pseudohermaphrodite. In the chick and the rat, complete alteration of the genital tract so that it has the morphologic structure of the opposite sex has been accomplished by the injection of steroid hormones during gestation.[7] The gonads have been said to be completely reversed in some experiments.[9]

Failure of differentiation of the interstitial or tubule cells can occur even in a normal hormonal environment. A defective germ plasm is postulated as the cause,[10] perhaps secondary to bad nutrition or disease of the mother during pregnancy.[11] Sterility or eunuchoidism may thus result; though the mechanism has not been fully established.

If adequate gonadal differentiation from the genital ridge has taken place, the descent to the scrotum offers the next threat to function and development. The anatomy and consequences of deranged descent are well understood and need no review. Bilateral cryptorchidism, or failure of the testes to emerge from the abdominal cavity, results in early, probably irreversible disappearance of spermatogenesis, if uncorrected, shortly after puberty has begun. This results from the higher temperature within the body as compared with that of the scrotal sac. Interstitial cell, or internal secretory activity, however, may persist for a longer period before failing. Emergence of the testes through the internal ring but failure to complete descent because of mechanical obstruction generally causes no disturbance in testicular function. The retractile testis descends at puberty and functions normally if left alone.

Occasionally, exploration of the cryptorchid individual reveals the presence of no testicular tissue, i.e., aplasia testis. A possible explanation for this defect is that the germ plasm is faulty and fails to provide an anlage for testicular differentiation. Another possibility is that the embryonic blood supply of the testes may have been compromised during descent, with complete testicular atrophy as a result.

Following normal development and descent of the testes, physiological control of testicular function is established through the gonadotropic activity of the anterior hypophysis. The testes remain small and immature until puberty. At this time, spontaneous hypophyseal activity occurs, gonadotropic hormone is secreted and causes growth and activity of both systems of the testis. This so-called gonadotropic hormone may be two distinct substances, one of which activates the interstitial cells and is probably identical with the luteinizing hormone of the female,[12] the other stimulates the tubule cells and is probably the same as the follicle stimulating hormone of the opposite sex.[13]

The individual identity of the two hormones in either sex is not yet universally accepted.

Gonadal failure in the young, without testicular destruction, obviously cannot be diagnosed until after puberty is normally initiated, since the infantile status is the norm before this event. Puberty may not commence until the sixteenth year of age in some healthy individuals, with an average age of onset at thirteen.[14] It is thus suggested that the diagnosis of gonadal failure should not be made before the seventeenth year of age unless cryptorchidism is present, or the increased amounts of pituitary gonadotropin associated with castration are found in the urine.[15] An understanding of the age limits of puberty will save many normal subjects, especially when somewhat obese, from false diagnosis and unnecessary treatment for testicular failure, e.g., eunuchoidism or Fröhlich's syndrome.

On the other hand, lack of pituitary function can prevent the onset of puberty. It is now suspected that the gonadotropic activity of the anterior hypophysis may fail to set in spontaneously at puberty and this is thought to be the cause of some cases of eunuchoidism.[16,17] Gonadotropic failure may result from compression or destruction of the pituitary by tumors, cysts, etc. Finally there may be cessation of pituitary function as the result of systemic disturbances such as inanition, uncontrolled diabetes mellitus, vitamin deficiency, or various diseases. The histological appearance of the gonads in the rat during inanition, for example, may approach that resulting from hypophysectomy, indicating that suppression of anterior hypophyseal gonadotropin output is the probable basis for the change.[18] This concept is strengthened by the response of these atrophied organs to gonadotropin.[19]

Spontaneous loss of testis function after puberty is said to occur. This produces the so-called male climacterium with symptomatology similar to that of the female menopause. There is some question as to the actual frequency of occurrence of this disturbance, as compared with symptoms due to a neurotic or emotional state at this age.

Destructive lesions of the testes, of course, may cause loss of testicular function at any time but usually after puberty. Tuberculosis, mumps, syphilis and tumors of various types affect the testes, though usually the involvement is unilateral. The remaining normal gonad prevents the appearance of castration symptoms or of sterility. If the remaining gonad is defective, castration symptoms of course, result. Injury with crushing or avulsion, *x*-radiation in excess, hernia repair with compromise of the cord in the scar and subsequent atrophy of the testes, and castration as for carcinoma of the prostate are other means by which gonadal function may be destroyed.

Persistent therapy with large doses of estrogen or androgen,[20] or failure of inactivation of normal amounts of these hormones in liver disease[21] increases the amount of circulating hormone and so may depress hypophyseal gonadotropin output. Spermatogenesis may be entirely repressed although this suppression is usually reversible on stopping therapy or on correcting the liver disturbance. Failure of internal secretory activity of the testes may result apart from tubule failure after hormone excess. This is not noticed during androgen therapy, due to the similar effects of the administered and endogenous hormones, but may be revealed during estrogen therapy when loss of libido may occur from suppression of hypophyseal secretion, and hence of testosterone.[22]

Destructive lesions of the anterior hypophysis from whatever cause obviously may result in changes in testicular function after puberty, just as in experimental hypophysectomy, and are similar in nature to the prepuberal causes of hypophyseal destruction. Aneurysm of the internal carotid artery

invading the sella turcica, adenomas of the hypophysis, and tuberculosis and syphilis involving the gland are usually adult, rather than prepuberal, complications.

Similarly, disorders of the remaining endocrine glands are more apt to occur in the adult and may affect the gonads, although whether directly or indirectly through the anterior pituitary is not clear. Toxic goiter may lessen libido and spermatogenesis,[23] and there may be a fall in urinary neutral 17-ketosteroid excretion.[24] Hypothyroidism may affect the testes to a marked degree, also with complete loss of function as a consequence.[23] Adrenal disease is also associated with gonadal difficulties.[25] This is true in Cushing's syndrome, now generally accepted as a disorder due to excessive adrenal cortical steroid effect,[26] though the primary disturbance may be elsewhere than in the adrenal. This disorder is associated with impotence and loss of testis function early in its course. Loss of adrenal cortical function, Addison's disease, usually does not cause striking testicular changes, although it may do so. Sterility may occur and the internal secretory function may fail in the acute stage of Addison's disease, to be restored upon restitution of blood pressure and of the blood sodium level to normal. This is shown by a decline in urinary neutral 17-ketosteroid excretion to zero in the acute phase, and a return to normal with recovery.[27]

In summary, gonadal failure may occur before or after puberty and may involve tubule or Leydig cell function or, as is usually the case, both. The cause of such disturbances may be genetic, embryologic, physiologic or pathologic in origin. Treatment is dependent on the primary disorder and its amenability to correction.

## CLINICAL ENTITIES

*Primary Prepuberal Gonadal Failure, or Eunuchoidism.* The symptoms and signs of this disorder are essentially those of failure to initiate or to complete the transition from the infantile to the adult status. Lack of testosterone effect explains all changes except sterility. Muscle weakness, obesity and unusual stature may be noted. Emotional problems may occur and usually result from the patient being aware that he is different from other boys of the same age. There is generally no evidence of spontaneous interest in the opposite sex and sexual activity is dormant.

Little has been added recently to the clinical descriptions of this disorder. The ultimate stature of the patient is unpredictable. One type of eunuchoid is classically stunted, probably due to failure of the puberal growth spurt which normally follows testosterone secretion by the maturing gonad. In these, the epiphyses usually fail to close and growth may occur, upon institution of replacement therapy, as late as at twenty-five years of age. Another type of eunuchoid is taller than normal. There is no adequate explanation for this except that the same phenomenon of overgrowth occurs in young castrates.[28] Body fat distribution varies considerably in different cases. Some tend to be unusually thin, others normal, and most tend to be moderately obese with a female body contour due to padding of the hips with fat and to occasional gynecomastia. The absence or markedly limited development of secondary sex characteristics, such as the voice, muscles, the youthful appearance due to lack of hair recession or loss, and the absence of graying, seborrhea, or acne are conspicuous and result from the lack of testosterone effect.[29] Later the skin ages prematurely. A slight degree of development of the penis and pubic hair occur frequently though these may be absent. This growth may come from slight testis secretory activity, or possibly from compensatory adrenal androgen output.[30] The testes are generally infantile but may

be somewhat larger, though flabby in consistency and insensitive to pressure. The prostate is generally impalpable. Ratios involving sitting height, standing height, length of arms, etc., are of interest to the anthropologist but have largely dropped out of clinical use.

The laboratory findings in this condition are of interest but are generally not pathognomonic. The introduction of the colorimetric method for determination of urinary neutral 17-ketosteroid excretion [31,22] led to the hope of objective diagnosis of testicular underfunction. However, the steroids of both the testis[33] and the adrenal cortex[34] are metabolized and excreted in large part as neutral 17-ketosteroids, roughly two-thirds of the amounts excreted coming from the latter and one-third from the former.[17] Thus a slight increase in adrenal activity may obscure a relatively large decline in testosterone output. Values in eunuchoidism vary from infantile to adult normal levels. (Table 1.)[35] Since systemic disease and especially disease of the pituitary, adrenal and thyroid glands may produce similar changes in 17-ketosteroid excretion,[36] this determination is not of diagnostic value. Testosterone therapy increases the urinary 17-ketosteroid output since the administered hormone is metabolized and excreted in the same way as is the endogenous hormone,[33] whereas methyl testosterone is not so metabolized and may decrease the urinary 17-ketosteroid excretion, probably by anterior pituitary depression. Partition of the total urinary 17-ketosteroids into alpha, beta, alcoholic and non-alcoholic fractions is possible[37] but no results have been reported in gonadal failure.

Estrogen seldom appears in the urine in this condition. (Table 1.) If present, it is presumably adrenal in origin[38] although other sources have been suspected. The urinary output of gonadotropin from the anterior pituitary is generally low. (Table 1.) Occasionally, it is at castration levels. (Table 1.) The low outputs have suggested, as mentioned above, that lack of anterior pituitary function may be the cause of testicular failure in some instances of eunuchoidism. However, another explanation for the low urinary titers of gonadotropin may be that enough testosterone is secreted by the inadequate testes to prevent a castration output by the hypophysis, but not enough to produce secondary sex changes.

TABLE 1
EXCRETION VALUES OF URINARY GONADOTROPIN, ESTROGEN, AND TOTAL NEUTRAL 17-KETOSTEROIDS IN A SERIES OF EUNUCHOID PATIENTS

| Patient No. | Urinary Gonadotropin M.U./24 Hours | Estrogen R.U./24 Hours | Total neutral 17-ketosteroids Mg./24 hours |
|---|---|---|---|
| 333 | | | 14.6 |
| 255 | 80 | less than 5 | 11.0 |
| 324 | 80 | less than 20 | 10.8 |
| 437 | less than 80 | | 9.3 |
| 377 | less than 60 | less than 5 | 9.3 |
| 183 | less than 10 | less than 5 | 9.1 |
| 132 | less than 5 | less than 5 | 8.6 |
| 188 | 40 | | 8.6 |
| 23 | less than 10 | less than 5 | 8.6 |
| 265 | 30 | less than 5 | 8.6 |
| 273 | 5 | less than 5 | 7.3 |
| 520 | | | 6.8 |
| 217 | 10 | less than 5 | 4.8 |
| 98 | 120 | less than 5 | 4.8 |
| 368 | 20 | | 4.3 |
| 249 | less than 5 | less than 5 | 2.6 |
| Normal range | less than 5-40 | less than 5-20 | 7.0–17.0 |

The blood count is altered in that the hemoglobin and red blood cell count approach childhood levels.[39] Basal metabolic rate determinations are generally in the low normal range. *X*-rays of the epiphyses usually show delayed fusion, even as late as twenty-five years of age. Creatin is generally present in the urine of the eunuchoid, not having disappeared at the usual age of puberty. There is decreased tolerance to in-

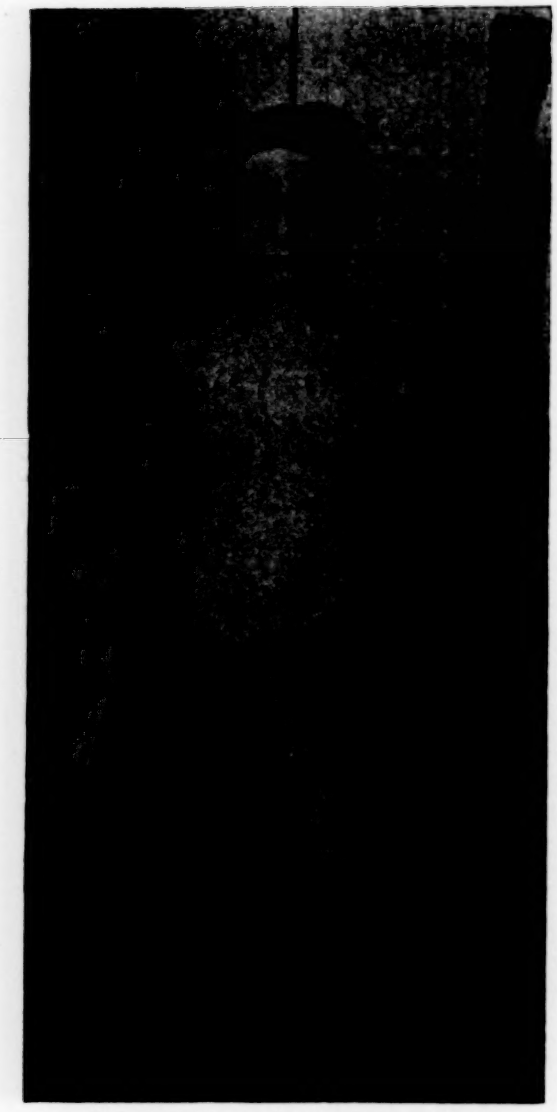

FIG. 1. Fröhlich's syndrome. The original case before operation on the hypophysis. (Reproduced from Fröhlich's original report.)[43]

gested creatin[40] as in childhood. Laboratory examination for sperm shows a scanty ejaculate, if any, which contains detritus and a few cells but no viable sperm. Testis biopsy affirms the absence of spermatogenesis and generally shows a change in the morphology of the Leydig cells which is difficult to interpret.[41a,b]

All the above laboratory changes are corrected by testosterone, except the absence of spermatogenesis and the histologic changes.

*Prepuberal Secondary Gonadal Failure due to Destruction of the Hypophysis, or Fröhlich's Syndrome.* In 1898, Babinski[42] described a syndrome of genital underdevelopment and moderate obesity in a girl, secondary to a tumor in the region of the pituitary gland and involving the hypothalamic area. One year later, Fröhlich[43] described the same clinical picture in a boy with a carcinoma of the pituitary gland. Since then the obese eunuchoid, and the healthy but obese boy whose sexual maturation has not yet clearly advanced have been confused with the picture described by Fröhlich. A glance at the picture of Fröhlich's original case (Fig. 1), shows the discrepancy between the original case and the subjects now diagnosed as instances of the syndrome. (Figs. 2 and 3.) Obese boys practically always initiate testicular function and mature spontaneously without glandular therapy if left alone[44] so that avoidance of unnecessary therapy in this group must be emphasized. Certainly, such therapy is not warranted before the end of the normal period for the onset of puberty, i.e., the sixteenth year.

Replacement therapy in Fröhlich's syndrome requires a potent anterior pituitary extract to take the place of the secretions of the destroyed gland. Clinically effective anterior pituitary preparations are not available but pregnant mare's serum hormone[45] has helped and methyl testosterone[46] provides very satisfactory relief. The primary intracranial lesion obviously must be treated as indicated.

*Postpuberal Primary Non-destructive Gonadal Failure—The Male Climacterium.* In about 40 per cent or more of male castrates, young or old, there are vasomotor symptoms similar to those of the female menopause[28,47]—flushes, sweats, muscle weakness. It is not unreasonable, therefore, that a spontaneous decline in gonadal function in the male has been postulated. It is said to occur at about the same age as in the female and is asso-

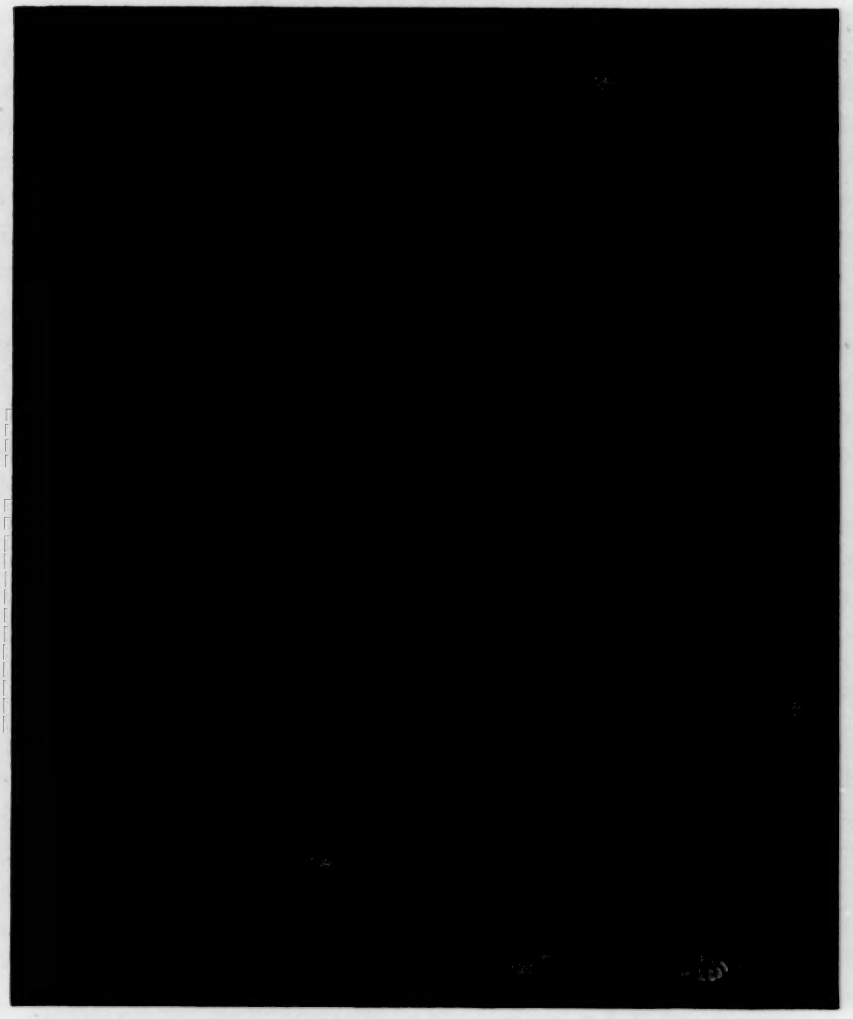

FIG. 2. A. F., No. 549845, age twelve years; untreated obesity; to illustrate the difference from true Fröhlich's syndrome with injury to pituitary and hypothalamus. (Fig. 1.)

FIG. 3. A. F., No. 549845, age seventeen years; untreated obesity; to illustrate spontaneous puberal maturation without therapy.[44]

ciated with similar symptoms as the female menopause, plus impotence and loss of libido.[48] The frequency of diagnosis of this syndrome varies greatly in different clinics. A clear explanation of this discrepancy is not at hand. It is certain, however, that the occurrence of instances of true spontaneous male climacterium decreases as more care is taken to rule out psychogenic and other disturbances.

A study of patients in the older age groups with carcinoma of the prostate indicates an almost universal betterment of the carcinoma after castration.[22] Serum acid phosphatase falls if previously increased. Since both growth of the carcinoma and the increased phosphatase level depend on testosterone, it is clear that some degree of testosterone secretion is almost universally present in older men beyond the age of climacterium. Thus it is implied that if the male climacterium is due to gonadal failure, it must be a reduction and not complete cessation of gonadal secretion (such as occurs in the female at the menopause) which has

caused the symptoms. Possible evidence for such a decline in function is found in the decreasing but persistent excretion of neutral urinary 17-ketosteroids with advancing age.[49,50] Study of the testis histologically after castration for carcinoma of the prostate shows poor correlation with the testicular function indicated by the clinical response to the operation.

Against the hypothesis that simple reduction and not complete disappearance of circulating testosterone is the cause of vasomotor symptoms, is the similar incidence of such symptoms in the old and in the young following castration. This indicates that whatever testosterone is still circulating in older men is still sufficient to prevent symptoms, despite previous decreases in rate of secretion. Were it not for the dangers of testosterone therapy, the point would be academic and suspected cases of climacterium could with impunity be given testosterone as a therapeutic trial. However, the malignancy of carcinoma of the prostate is increased by such treatment and spermatogenesis is arrested. Moreover, when a psychoneurosis and not gonadal failure is the cause of loss of libido and impotence, the psychological disorder tends to become fixed by spectacular and repeated therapeutic efforts and the subsequent chance of psychotherapeutic efforts being effective is lessened. All in all, the author believes that the syndrome of spontaneous male climacterium is not common. Certainly objective confirmation of the diagnosis should be obtained when possible by demonstration of castration levels of hypophyseal gonadotropin in the urine.

*Secondary Gonadal Failure in Cirrhosis.* Testicular atrophy and cessation of spermatogenesis is a common pathological finding in cirrhosis of the liver. The usual symptoms of testicular failure, however, i.e., loss of libido, impotence, sterility and muscular weakness are often lost sight of due to the predominance of more serious symptoms. The scanty secondary sex-hair of these people may conceivably be due to testicular failure but is more probably due to a constitutional factor. The mechanism for this disturbance may be two-fold: First, gonadotropin output of the anterior pituitary is sharply restricted in malnutrition[19] and in thiamin deficiency.[51] Second, inactivation of free circulating estrogen by the cirrhotic liver is interfered with[21] both because of actual loss of liver parenchyma and because of the absence of thiamin, which appears to be necessary for the liver cells to perform this function optimally.[52] Thus, estrogen arising from the adrenal in the male may accumulate in the blood, depress the anterior pituitary, and so result in testicular atrophy. Testosterone inactivation by the liver does not appear to be affected in thiamin deficiency although obviously, serious liver parenchyma destruction will lessen the ability of the organ to inactivate this hormone also.

*Secondary Gonadal Failure in Other Systemic Diseases.* Although the signs and symptoms of testicular failure may occur in many systemic diseases, just as in cirrhosis, the symptoms of the major disease generally obscure those arising from testicular failure. Correction of the underlying disease results in restoration of testicular function. Thus proper diagnosis and not gonadal replacement therapy is indicated in most instances. In the laboratory, gonadotropic and urinary neutral 17-ketosteroid excretion are moderately depressed, generally not absent.[53]

*Sterility.* Testicular failure, from whatever cause, usually involves both the spermatogenic and internal secretory functions, with resultant sterility. Proper diagnosis and correction of the original disorder, when possible, usually results in spontaneous return of sperm production to normal. Alcoholism is listed as a systemic cause of cessation of spermatogenesis but the asso-

ciated malnutrition and vitamin deficiency probably account for the effect on the testis rather than a direct toxic effect of the drug.

A group of cases with testes of normal size and with normal secondary sex characteristics, but almost no sperm production, deserves mention.[54] There is no demonstrable cause for the failure of spermatogenesis nor is hormonal treatment effective. One possible explanation for the aspermia of this group may be some genetic fault in tubule cell differentiation and function.

*Impotence and Premature Ejaculation.* These disorders are almost always psychogenic rather than gonadal in origin when definite organic change is not evident. The only objective criteria for a testicular origin of these symptoms would be the demonstration of aspermia and of castrate amounts of pituitary gonadotropin in the urine. Even then, after true castration, the effect of the psyche is large since not all patients lose their potency[55] and premature ejaculation is quite uncommon if libido is preserved.

## TREATMENT

The goal of treatment in testicular as in other disorders is to establish an etiologic diagnosis and to correct the basic disturbance when possible. In most instances, gonadal failure in man is secondary to a systemic disorder which is correctible, such as toxic goiter, malnutrition, cirrhosis, etc., and no specific treatment for the testicular failure is indicated. In primary gonadal failure, or eunuchoidism, on the other hand, the sole problem is treatment of the testicular failure and the aim of therapy is to stimulate the testes. This is not always possible, so that replacement therapy is often necessary. This latter corrects the symptoms and signs of testosterone lack but does not affect the aspermia.

As stated above, recent work suggests that more, rather than fewer, cases of eunuchoidism are due to lack of anterior hypophyseal gonadotropin secretion. A program of therapy to stimulate the testis has been devised[64] using chorionic gonadotropin from human pregnancy urine and a specially prepared, not yet generally available, pituitary follicle-stimulating substance. This has produced spermatogenesis histologically, as shown by testis biopsy and has caused development of secondary sex characters in these patients.[16,55,56] However, sperm examinations and fertility results following such therapy are not yet reported. The chorionic gonadotropin has a luteinizing or interstitial cell stimulating effect, while the anterior pituitary extract is follicle or tubule stimulating. No so-called antihormone response[57,58] results from treatment with human-derived hormone since the material is not a foreign protein and so does not cause the production of an antibody. However, animal-derived anterior pituitary extracts are antigenic and so are useful for short periods only.[59]

Gonadotropin therapy, to be successful, must be given by injection daily, or three times weekly. A dosage of 1,500 I.U. of chorionic gonadotropin together with an experimentally determined amount of follicle-stimulating material, depending on the preparation, is given for some weeks. Pregnant mare serum hormone has been used for short periods, instead of the hypophyseal follicle stimulator.[16]

When the testes are missing, or when the above program is inadvisable or has failed, replacement therapy with testosterone is employed and, except for lack of sperm production or increase in testis to normal size, is completely successful. This therapy, as with insulin in diabetes, must be continued and adequate dosage must be given. Suboptimal dosage may produce little or no visible changes and thus lead to the discontinuance of a therapy which is always successful when sufficient hormone is administered. The illustrations from a series

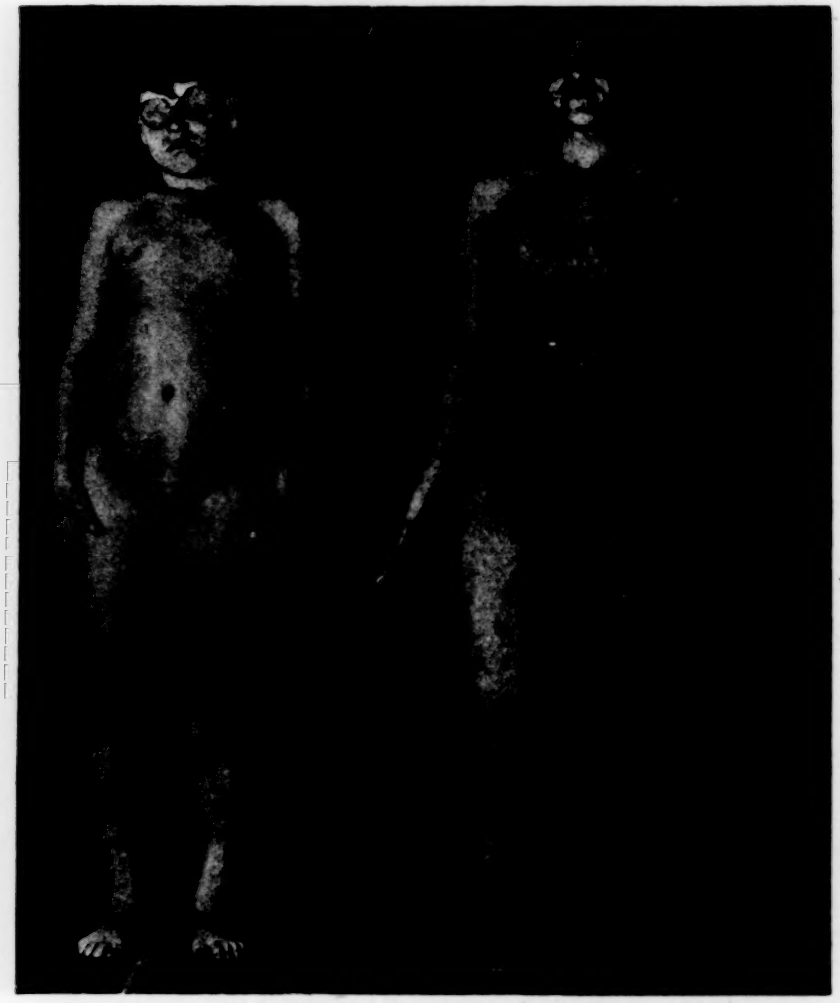

FIG. 4. A. P., No. 562365, age sixteen years; aplasia testis, untreated.

FIG. 5. A. P., No. 562365, age twenty-one years; aplasia testis after male hormone therapy for five years.

of patients treated for three to six years in the Presbyterian Hospital and Thyroid Clinic of the Vanderbilt Clinic (Figs. 4 to 7) indicate the results obtainable with adequate therapy.

Methyl testosterone, as androgen therapy, is used exclusively in most clinics because it can be given by mouth. As stated, the substitution treatment of eunuchoidism is chronic. Continued injections of testosterone propionate in oil, 0.025 Gm. three times weekly, and repeated implantations of pellets, absorption from which is uncertain, are inconvenient and do not seem justifiable except temporarily. Free testosterone and testosterone propionate are largely inactivated by mouth, unlike the methyl derivative, and so the oral route is not available. Sublingual administration is used but is uncertain and is inefficient if the material is not retained long enough before swallowing. Methyl testosterone meets these objections. It is effective by mouth and its effects are identical in every way with those of testosterone except for the production of an intense creatinuria[60,61] in the usual dosage of 0.03 to 0.06 Gm. per day. No toxic symptoms have been reported.

Recently, however, the suspicion that

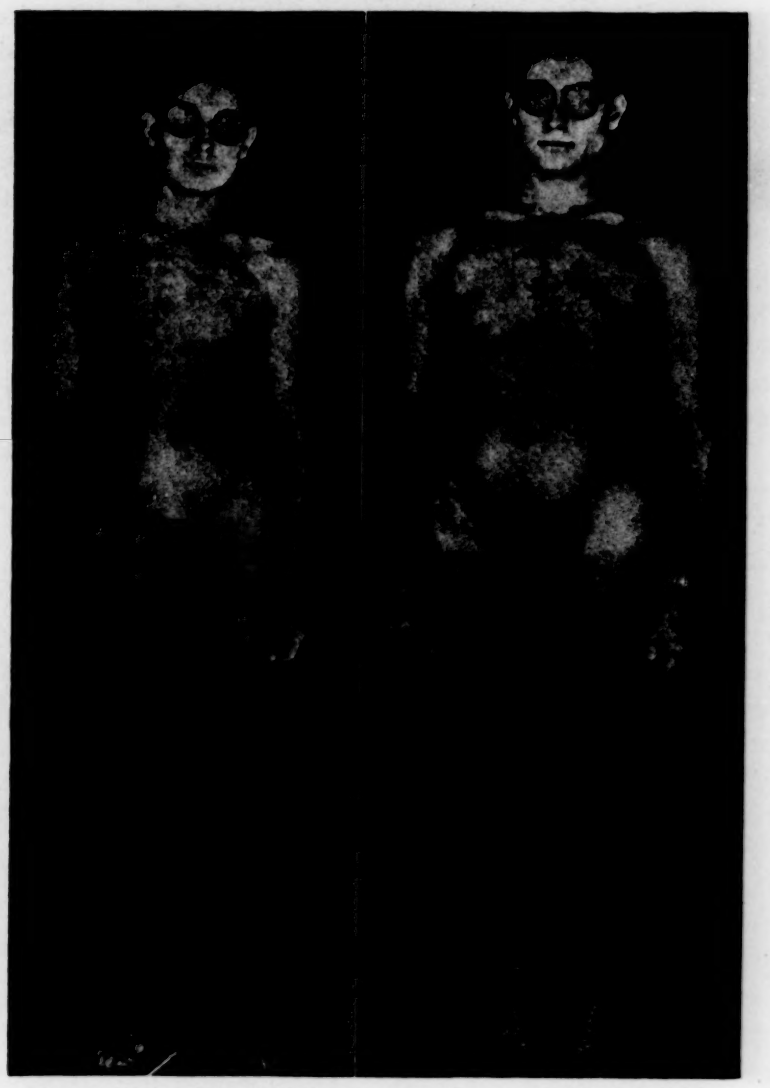

FIG. 6. A. L., No. 567231, age twenty-five years; eunuchoidism, untreated.

FIG. 7. A. L., No. 567231, age thirty years; eunuchoidism, after male hormone therapy for five years.

jaundice may be produced by methyl testosterone has been raised in three cases in the author's series.* The clinical picture was the same in all three cases, that of hepatitis with a peculiar protracted course and with a low blood alkaline phosphatase and a repeatedly negative cephalin flocculation test. There is an early obstructive phase with bile in the urine but not in the stools. Six other cases of jaundice associated with methyl testosterone have been cited to the author* but in only one case† was the drug given a second time, upon which there was a recurrence of jaundice. The other five instances might have been instances of intercurrent infectious hepatitis.

The results of adequate testosterone administration are striking, and the transformation from the infantile state to adulthood is similar in every respect to that during

* In a fourth case seen recently, jaundice did not recur with a retrial of the drug.

* Cases of Drs. Reid R. Heffner, New Rochelle, N. Y., Joseph Eidelsberg, New York, N. Y. and E. Perry McCullagh, Cleveland, O.

† Case of Dr. Reid R. Heffner.

normal puberal maturation. Muscle development is promoted, there is gain in weight and often an increase in height. In one case (Fig. 5) there was a gain of 76 pounds and 11 inches in five years. Secondary sex hair appears during therapy but its amount and distribution are influenced by hereditary and constitutional factors. Penis, prostate and seminal vesicle growth, and evidences of sexual activity are all promoted to the appropriate chronological level. Marriage is undertaken and consummated in most cases.

Acne appears early and may be severe and annoying. A slightly reduced dosage helps this condition, although most patients resist any attempt to lower dosage. Once treatment is started, complete cessation of the hormone therapy may result in marked muscular weakness, leg pains, loss of libido and erections and an anxiety state. These are instantly corrected by renewal of the drug.

In the castrate and male climacterium patients, successful replacement therapy is possible with methyl testosterone 0.03 and 0.06 Gm. a day just as in the eunuchoid group. A combination of methyl testosterone and estrogen, e.g., stilbestrol, has possibly been more effective than androgen alone in restoring strength and alleviating vasomotor symptoms.

Testosterone therapy is often given to men with adequate testicular function but with psychogenic loss of libido or impotence, or with misdiagnosed male climacterium. Repression of spermatogenesis, activation of carcinoma of the prostate as shown by increase in the acid phosphatase in the blood, or the exaggeration or fixation of neurotic trends are possible consequences of such therapy, which cannot be considered lightly.

The emotional problems of patients with testicular failure are important.[62,63] The borderline eunuchoid may be suffering from psychogenic impotence in addition to his physical difficulty. The loss of libido is then not entirely correctible by adequate replacement therapy. Here the hormonal drive is adequate but the emotional block prevents adequate activity or direction to the stimulus, the same mechanism that disturbs the physically normal male with psychogenic impotence. Obviously, psychiatric help from trained sources is indicated.

In summary, poor response to replacement therapy with male sex hormone generally suggests that the diagnosis must include sites of disorder other than the testes proper. It should be emphasized that unless there is certain evidence that gonadal failure exists as a result of primary testicular or pituitary failure, testosterone or gonadotropin therapy is unnecessary, possibly harmful and probably futile. Cure of a primary disease originating outside the testis, if such a disease is present, is all that is necessary to treat the secondary gonadal difficulties.

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# Seminar on Thromboembolism

## Mechanism of Blood Coagulation*

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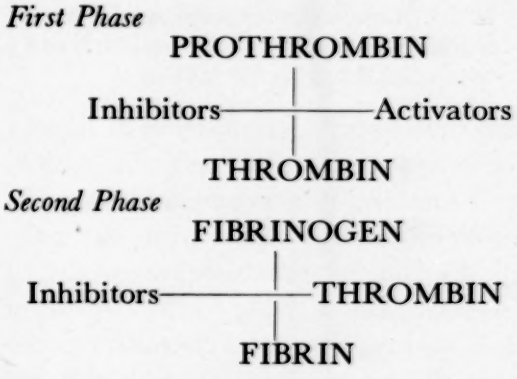
BLOOD coagulation is essentially a series of colloidal chemical reactions[13] normally proceeding only after blood is shed, but also occurring pathologically[15] within the vascular system as a factor in thromboembolism and in blood or plasma extravasations (fibrinous exudates).

*Nature of Clotting Mechanism.* The logical approach to an understanding of the mechanism of coagulation along strictly biochemical lines is rendered difficult by the complexity of agents involved.[14] Water, pH, salts (e.g., Ca ions), proteins (e.g., fibrinogen and fibrin, prothrombin and thrombin), fatty substances (e.g., the phospholipids and cephalin), carbohydrate derivatives (e.g., heparins) and a complex system of proteolytic enzymes are all clearly implicated and their modes of interaction extend into the farthest reaches of chemistry, including some highly specialized divisions of physical, colloidal and enzyme chemistry. Nevertheless, as a result of carefully controlled *in vitro* experimentation, the basic mechanisms of clotting are now fairly well understood and can be presented in a straightforward manner if the logic of the methods of study is comprehended.

The clotting processes, which are largely simultaneous in ordinary coagulation of the blood, are primarily plasma phenomena that can be separated experimentally into two phases:




Fibrin is the essential material of the clot. It is deposited as a semisolid quasicrystalline "gel" when the colloidal solution of its plasma protein precursor, fibrinogen, is acted upon by a specific coagulant, normally thrombin. It is only necessary to inject a potent thrombin solution intravenously into an experimental animal and to observe the prompt intravascular coagulation (thrombosis) to confirm the rather obvious fact that active thrombin is not normally present in the circulating blood. What the plasma does contain is a protein precursor, prothrombin, which can be isolated and made to yield thrombin by appropriate activation procedures.[32] Ordinarily, these coagulation reactions are subject to a variety of inhibitory mechanisms, many of which can be elucidated *in vitro*.[29]

*Nature of Fibrin Formation.* The typical (quasicrystalline) microscopic "needles" or "filaments" of fibrin, best seen under dark-field, are shown in Figure 1. The cited

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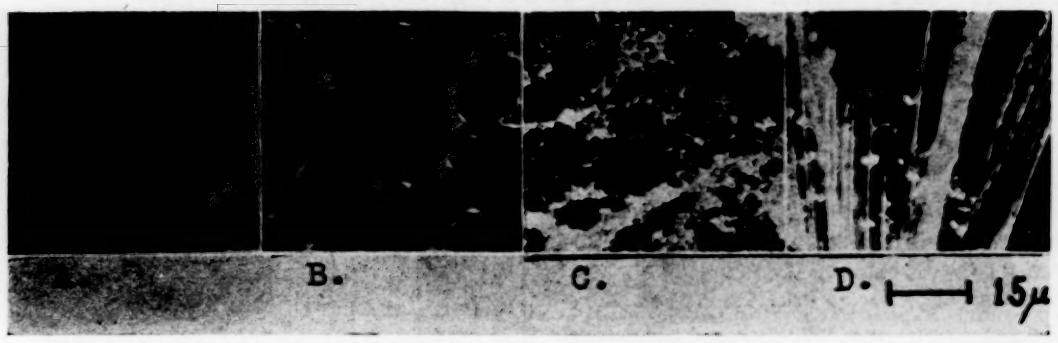



FIG. 1. Dark field microscopy (oil immersion lens) of fibrinogen (dog) mixed with A, tryptase-free thrombin; B, crystalline papain; C and D, ninhydrin. (FERGUSON, J. H. and RALPH, P. H. *Am. J. Physiol.*, 138: 648, 1943.)

experimental data[11] demonstrate a thrombin-like action of crystalline papain (which, unlike thrombin, is a proteolytic enzyme) but differentiate true fibrin from the non-descript fibrinogen denaturation produced by ninhydrin and a wide variety of other chemical agents. The exact chemistry of the thrombin-fibrinogen interaction is not yet understood. It is known that fibrinogen molecules, despite a high molecular weight (about 500,000),[5] are extremely attenuated or filamentous, so that in a moving fluid they tend to orient themselves "like logs in a stream." If the logs can be made to pile up in a criss-cross manner they tend to form a mass which blocks the stream. This rather crude analogy can be presented in a technical nomenclature ("coacervation")[26] as a basis for a fundamental explanation of fibrin gel formation and its rôle in thromboembolism. The underlying forces and particularly the part played by thrombin have still to be worked out. Facts,[15] relevant to the suggestion that electrochemical forces participate, include the difference between isoelectric points (pH) viz., fibrinogen 5.4, thrombin 4.4. The very minute amounts of thrombin needed and certain other data are compatible with the view that thrombin is a special type of enzyme.

*Experimental Study of Clotting Reactions in vitro.*[10] Despite considerable progress[5] in recent years, few of the chemical agents concerned in the clotting process can be isolated and determined with quantitative precision. Ordinary analytical methods especially fail to detect traces of impurities which may markedly modify behavior in clotting tests. For such reasons, it is desirable to comment briefly upon a technic for circumventing these difficulties during the study of the basic clotting reactions *in vitro*.[15]

*Conditions Affecting Coagulation.*[31] The colloidal reactions are influenced by (1) temperature, (2) pH, (3) salt concentrations, (4) concentration (dilution) of specific factors, (5) adsorption and other colloidal phenomena. In the last category are the well known effects of "wettable" surfaces (e.g., blood clots more easily in glass than in paraffined, plastic or silicone-treated tubes) and the clot-aiding or "fibrinoplastic" (second phase) effect of gum acacia and a variety of non-specific adsorptive colloids. The first step, therefore, is to standardize these experimental conditions.

*Purity of Reagents.* Secondly, while it is desirable to employ isolated reagents as pure as possible, it is even more important to test each reagent and combination of reagents to rule out any significant effects of impurities which can modify the clotting tests.

*Timing Reactions.* Finally, the reactions are carefully timed with reference to a specific end point, especially clotting time (c.t.). By confining the experimental analy-

ses to similarly constituted mixtures of the same batch of reagents, uncontrollable variables are minimized and the data obtained assume a quantitative as well as qualitative significance. The examples of Figures 2 and 3 illustrate the above points.

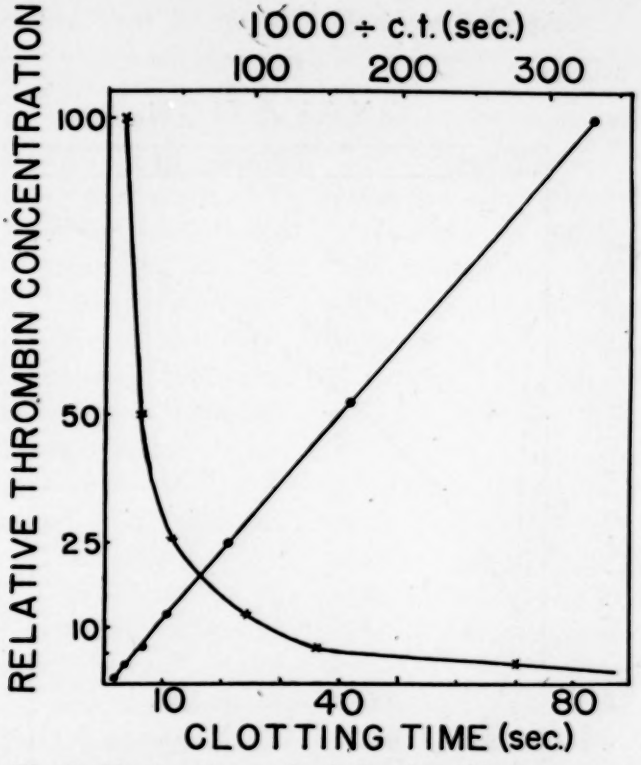



FIG. 2. Clotting time and relative thrombin concentration (percentage). The inverse law: Clotting times (sec.) and 1000 ÷ c.t. (sec.) of 1.0 cc. fibrinogen (bovine, 1:200) + 0.25 cc. thrombin (bovine, 1:500); borate buffer (pH = 7.7); temp. = 25°c.

*Clotting Time and Relative Thrombin Concentration* (*Fig.* 2). When a series of thrombin dilutions is tested on a given fibrinogen solution, it is easy to observe that the stronger the thrombin the shorter is the clotting time. The strength of the fibrinogen is of very minor importance between 1.0 and 0.2 per cent. Under somewhat limited experimental conditions, the c.t. is inversely proportional to the thrombin concentration and the latter may be expressed as percentages or units in terms of an empirical dilution technic. It is quite unnecessary to go into technical details in order to grasp the above stated basic fact that a shorter clotting time (under standardized conditions) means more thrombin; this fact may be applied in numerous practical ways.

*Prothrombin Activation Curves* (*Fig.* 3). When a prothrombin solution, mixed with suitable activators (v. below) is sampled at successive time (incubation) periods and the clotting time of each measured sample added to a test fibrinogen is noted, the graphic plot of the data obtained yields what we call "the prothrombin activation curve." Again the technicalities with reference to rate and amount of thrombin formation are subsidiary to the simple indication of increasing amounts of thrombin as detected by the shorter clotting times. When the curve levels off at the shortest c.t. the activation under the prevailing conditions is 100 per cent complete.

The factual statements in the following paragraphs are supported by experimental modifications of the simple methods we have just outlined.

ACTIVATORS OF PROTHROMBINS[2]

The factors which participate in the conversion of prothrombin to thrombin may be considered each in turn.

complete thrombin formation. Oxalates or citrates, etc., depress the ionization of calcium. Added early enough (Zone I of Figure 3) they can completely prevent the activation of thrombin. Added too late, the thrombin being fully formed (Zone III of Figure 3), they are unable to prevent clotting and have only a very minor non-specific

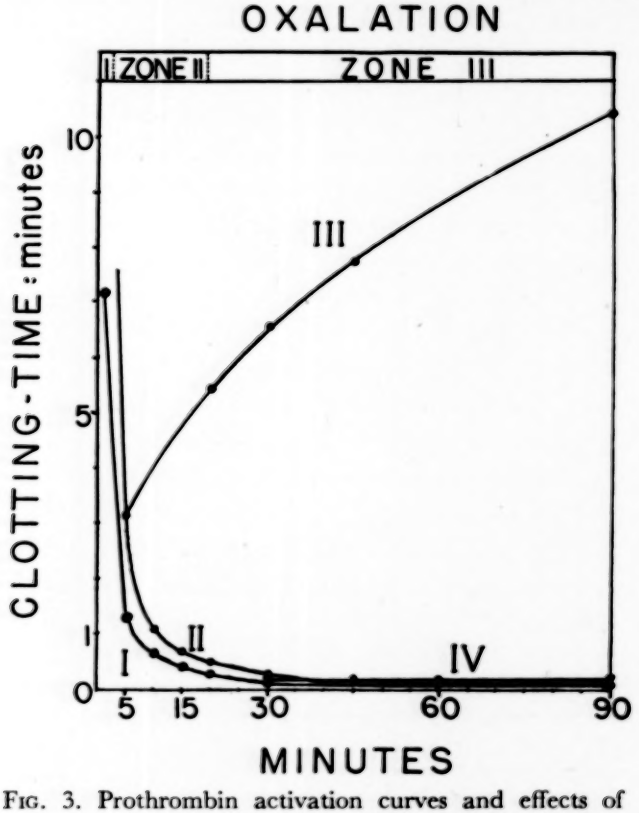



FIG. 3. Prothrombin activation curves and effects of oxalation. II, ⊙—⊙ prothrombin (bovine, 1:200) + brain thromboplastin + M/10 $CaCl_2$; clotting times of 0.25 cc. sample + 0.25 buffer + 1 cc. fibrinogen (25°C.); II. •—• same mixture tested on oxalated fibrinogen; III. •—• same mixture oxalated after five minutes and tested at intervals on fibrinogen. (Volumes of thrombic mixture and oxalate in clotting test same as in II;) IV. x—x same mixture oxalated after thirty minutes and tested as was indicated in step III.

*Calcium.*[8] Calcium ions ($Ca^{++}$) are ordinarily essential for the first phase of the clotting mechanism. They are not needed for the second phase (thrombin-fibrinogen interaction) and an excess is inhibitory owing to certain non-specific salt effects. Too little calcium results in slow and in-

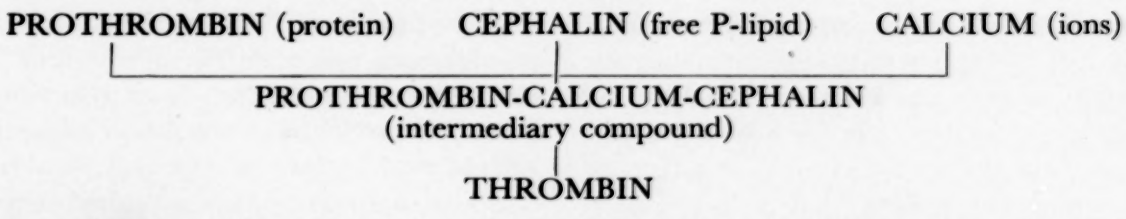
clot retarding effect in excessive concentrations. There is, however, an intermediate zone (II) during which a large excess of oxalates (or citrates) can progressively inactivate the thrombic mixture. Re-addition of calcium restores the activation. This we explain as evidence for the existence of a

calcium-containing *intermediary complex* (with prothrombin and phospholipid) existing briefly during thrombin formation (v. infra). The final thrombin may be obtained free of calcium by oxalation and electrodialysis.

In the normal plasma or serum there is about 10 mg. per cent of calcium. More than half of this is ionized and the rest is in protein-bound and other non-available forms. When blood is oxalated or citrated and subsequently recalcified there are a series of equilibrations between ionized and bound forms of calcium and the time factors involved are nearly as important as the amounts. The following statements are supported by experimental evidence and have direct clinical significance: (1) the normal blood $Ca^{++}$ level is about optimal for coagulation; (2) the minimum $Ca^{++}$ requirement for clotting is well below any level encountered in the severest hypocalcemias (e.g., tetany); (3) clinical hypercalcemias and attempts to raise the blood calcium therapeutically do not significantly modify the clotting process. The only possible clinical application of the *in vitro* data on calcium and coagulation is suggested by recent reports[21] that calcium is a variable in the prothrombin clotting time (p.c.t.) test (v. infra) especially in the hypoprothrombinemia due to dicumarol which is one of the modern methods of treating and preventing thromboembolic conditions. We except, of course, the important use of citrates for blood transfusions, etc.

*Thromboplastic Factors.* It is possible, though with difficulty, to prepare prothrombin solutions which are not activated by calcium salts alone, or only very slowly and poorly. There is evidently need for an additional factor or factors for which the descriptive term "thromboplastic" is widely used.[14]

*Phospholipid.* Cephalin (phosphatidylethanolamine) is a phospholipid substance which can be isolated by chemical means from plasma, cells and tissues (especially the brain). It has no direct action on fibrinogen or on prothrombin alone, but in the presence of $Ca^{++}$, it is a thromboplastic agent demonstrably active even in dilutions of 1:1,000,000. Inadequate amounts, however, result in slow and incomplete thrombin formation. Optimal amounts complete the conversion of prothrombin to thrombin in a very few minutes. The final thrombin may be obtained free from phospholipid. There are still technical difficulties in obtaining phosphatide-free prothrombin, however, so that we can at present merely point to experimental evidence that cephalin usually participates in thrombin formation only when available in the "free" state, as compared with protein-bound forms in which the cephalin is not available. Like calcium, therefore, the phospholipid appears to act via an intermediary complex or compound. These views are summarized as follows:




Although data are negative for other currently recognized phospholipids, there are a few suggestions pointing perhaps to as yet unidentified thromboplastic agents of this class.

*Thromboplastin (Thrombokinase).* The long observed fact that the fat-soluble phosphatides are not as potent as the similarly acting crude, watery tissue extracts justifies

the continued use of names like thromboplastin or thrombokinase for the latter. A macromolecular protein complex containing phosphatides[3] has recently been isolated from lung extracts and is said to be an extremely potent thromboplastic agent. It is also said to lack demonstrable proteolytic properties such as are frequently encountered in crude aqueous tissue extracts, including most commercial thromboplastins.

In general, thromboplastins require $Ca^{++}$ but differ from cephalin in three important respects, namely, (1) greater potency, (2) effectiveness in presence of heparin (v. infra) and (3) greater ability to restore clotting time to normal when added to hemophilic plasma. The defect in hemophilia is certainly related to the thromboplastic system.[29]

*Tryptase (Plasmin or Fibrinolytic Enzyme).* The additional thromboplastic actions of thrombokinase can be imitated *in vitro* by simply adding a small quantity of crystalline trypsin (pancreatic enzyme)[7] to the ordinary Ca + cephalin activator system. Trypsin also resembles thromboplastin in the dangers of intravascular coagulation and shock-like phenomena which follow intravenous injection and preclude any such use *in vivo*. With great care, perhaps aided by the protective action of natural trypsin-inhibitors in the blood, one or two hemophilic patients[34] were recently found to tolerate enough trypsin intravenously slightly to lower their clotting time for a brief period. These data are of no clinical significance but support the idea that a proteolytic enzyme resembling trypsin may play a rôle in blood clotting. We have experimental data to suggest that trypsin is not a thromboplastic agent in its own right but merely a factor which catalyzes the actions of cephalin and calcium when thrombin is being formed in the presence of interfering proteins. Obviously, this could be of great importance in the natural clotting system because of the occurrence of trypsin-like enzymes in the blood.[12]

These enzymes have been recognized since the turn of the century and are often called fibrinolysin or fibrinolytic enzyme, when studied in connection with fibrinolysis or digestion of fibrin, a phenomenon which occurs to a variable degree in natural blood clots.[27] The term tryptase[12] merely means trypsin-like (as opposed to the cathepsins and possibly other types of blood proteases) referring especially to alkaline pH optimum ($7.5 \pm 0.5$), ability to attack a variety of ordinary protein substrates (casein, gelatine, hemoglobin, fibrin, fibrinogen, etc.,) and susceptibility to ordinary trypsin-inhibitors. It differs from pancreatic trypsin, however, in (1) origin, (2) specific activator (kinase)[22] and (3) other important ways.[4] For these reasons, the new name, *plasmin* has been suggested[4] but perhaps somewhat prematurely. Our strongest reason for preferring tryptase is to emphasize the numerous analogies to the pancreatic enzyme. For instance, the plasma enzyme resembles its prototype[28] in (1) inactive precursor (tryptogen), (2) need for a specific activator (tryptokinase, e.g., streptokinase, miscalled "streptococcal fibrinolysin"[22]), staphylokinase and similar activators of bacterial origin), (3) inhibition of active enzyme (anti-tryptase) and of the kinase (anti-tryptokinase, miscalled "antifibrinolysin").[22] Antitrypsins (crystalline) from pancreas[9,18] and soybean[25,35] have certain anticoagulant effects.

Some very recently published experiments[17] afford clear proof of the trypsin-like thromboplastic action of natural tryptase from a variety of plasma protein preparations. For both enzymes it must be emphasized, this clot aiding effect requires much smaller amounts of enzymes than are needed for ordinary proteolytic effects, including fibrinolysis, fibrinogenolysis (which we use for an enzyme assay method sensitive

to 1:1,000,000 of standard trypsin), prothrombinolysis, etc. These proteolytic phenomena are essentially independent of the clotting mechanism but do encroach upon the coagulation problem at several points, e.g., (1) in the preparation of the protein clotting factors (the protease impurity is very difficult to get rid of); (2) clot-retraction and fibrinolysis (since pure thrombin-fibrin is a stable gel for weeks at 37°c., clot-retraction and fibrinolysis obviously require an additional factor and this is readily supplied by the addition of trypsin or tryptase-containing materials, including platelets); (3) fibrin resolution in the body, e.g., liquefaction and removal of thrombi and other fibrin clots, resolution of fibrinous exudates, liberation of emboli from thrombi formed in the circulatory system, etc.; (4) non-coagulability of cadaver- and menstrual-blood because of proteolysis of clotting proteins.[12]

*Clot Inhibitors.*[30] Normal serum has a considerable capacity for neutralizing active thrombin both *in vitro* and *in vivo*. The time-honored use of the term antithrombin has never justified itself by an adequate identification of the factor or factors involved.[14] Only one natural agent of clinical importance in this regard has yielded to biochemical attack but it is still imperfectly understood.

*Heparin(s).*[1] The singular is used for convenience but really represents a class of substances having the general composition of mucoitin polysulfuric esters (i.e., complex carbohydrate derivatives) the molecular building stones of which are (1) glucosamine, (2) glycuronic acid, (3) acetic acid (? var.) and (4) ester-linked sulfuric acids. Heparin is believed to originate in the metachromatic-staining, water-soluble granules of the tissue basophils or Ehrlich "mast" cells and to enter the blood stream, particularly in the liver, in small amounts normally but in considerable quantities in certain conditions, e.g., anaphylactic shock.

Heparin inhibits clotting both *in vitro* and *in vivo*. It acts on the two phases of the clotting system in a manner too complicated to admit of simple description but the most important point is the need for some "co-factor" (heparin-complement or proantithrombin) the nature of which is obscure. This is best supplied by the crude "albumin" fraction of plasma or serum.[30] Heparin plus a co-factor is able to prevent the clotting of fibrinogen by thrombin (when either factor alone has very little effect). Even smaller amounts of heparin plus a co-factor suffice to prevent the formation of thrombin from prothrombin. This action may be termed antiprothrombic but the best data suggest that it is chiefly directed against the thromboplastic mechanism (i.e., "antithromboplastic"). The actions of heparin can be neutralized *in vitro* and *in vivo* by the basic protamine, salmine.

*Platelets and Blood Clotting.*[36] Heparin also lessens the agglutination and breakdown of the blood platelets but so do most agents which arrest the coagulation process. The significance of this correlation is not yet clear but raises possibilities that some factor may be common both to coagulation and to platelet lysis. Could it be tryptase enzyme? Platelet preparations are thromboplastic, like most cellular and tissue extracts.

This and a variety of other observations have led many observers to give the platelets a part in the normal clotting mechanism which it is very doubtful that they deserve. Since platelet-free plasma undoubtedly clots readily on simple recalcification and is, potentially at least, the source of all the known clotting factors, and because the relative quantity of platelets and any agent (e.g., thromboplastin) they contain is so small relative to the plasma in which they are suspended, it is difficult to see how platelets can make any significant contribu-

tion to ordinary clotting. The clotting time is normal in thrombocytopenic purpura.[29] The rôle of agents of (damaged) tissue origin must also be regarded as accessory to the normal plasma clotting mechanism.

*Initiation of Clotting in Shed Blood and Intravascularly.* When the question is raised as to what we really know of the reasons for the normal fluidity of the circulating blood and for the occurrence of clotting when blood is shed or intravascularly (e.g., in thromboembolism), even the recent advances in our knowledge of the underlying mechanisms are inadequate without a little guesswork in the explanation. That this is risky is borne out by the failure of the theories of the late Professor W. H. Howell,[20] long the classical teaching in the United States. The chief failures of the Howell theory are (1) the inadequate development of the inhibitory idea, e.g., Howell's concept of some inhibitor + active agent (antithrombin-prothrombin) combination released from the inhibition by thromboplastin, with the prothrombin then yielding thrombin through the agency of $Ca^{++}$ alone, is not in accord with modern experimental facts; (2) inability to explain the initiation of thromboplastic action except by bringing in platelet and tissue-factors which the experimental evidence and the above mentioned considerations show to be unnecessary.

We are still in the position of having to explain the normal absence of intravascular clotting in terms of lack of active thrombin due, in turn, to non-operation of potential thromboplastic mechanisms. The modern work does, however, clearly prove the direct participation of thromboplastic factors in prothrombin formation (Ca alone is not enough). The basic explanation of clotting must be that the inactive precursor prothrombin (existing as such) requires thromboplastic activation and the non-availability of the latter is the crucial point. Two possible non-availabilities are suggested by our modern work; first, the non-availability of cephalin in ordinary protein combination. We suggest that the tryptase enzyme is important in mobilizing the phospholipid. The second is the non-availability of tryptase as long as it is in precursor (tryptogen) form and in the presence of antitryptase inhibitors. Our guess[12] is that the ordinary colloidal disturbances ("wetting," adsorption, etc.) when the blood is shed or when it contacts damaged vessel walls or tissues could cause activation of tryptase. This in turn mobilizes the phospholipid and the whole process of prothrombin activation until there is enough thrombin to clot the fibrinogen, either locally (e.g., in mural thrombus formation) or throughout the volume of blood or plasma. The cellular, especially platelet, factors in thrombus formation are probably an important accessory mechanism in thromboembolism since their agglutination and adhesion to damaged endothelium precedes true clotting and could add appreciable thromboplastic factors at the focus where the clot is initiated. There is obvious need for additional experimental knowledge before these views can be regarded as more than a working hypothesis.

The inhibitory mechanisms must be important in the control of each stage of the above series of reactions, viz. (1) inhibitors of the formation and of the action of the tryptase enzyme,[22] (2) inhibitors of the thromboplastic mechanism, preventing thrombin formation and (3) inhibitors of active thrombin. These may be regarded as successive lines of physiological defense against the untoward occurrence of coagulation of the blood *in vivo*. Experimental data seem to permit the generalization that these inhibitions are more significant as mechanisms for delay than for completely arresting the phenomena in question. Quantitative interrelationships are important.

## PHYSIOLOGICAL CONSIDERATION OF BLOOD CLOTTING

The fundamental mechanism of blood clotting yields to approach from the biochemical point of view. The physiological approach to the subject unites with clinical applications in pointing out the way in which the body can control the various factors involved in both health and disease.[29] Fibrinogen[19] and prothrombin[2] are plasma proteins comprising, according to latest estimates, 0.28 Gm.[5] and 20 mg.,[32] respectively, per 100 cc. plasma. The liver is of special significance in the metabolism of the plasma proteins and there are numerous experimental and clinical data to show that severe liver dysfunction or damage results in lowering of the plasma levels of these proteins with a concomitant bleeding tendency associated with failure of the clotting mechanism. Fibrinogenopenias are rarely significant but hypoprothrombinemias are quite the most common coagulation defect encountered clinically. Considerable practical value attaches, therefore, to the prothrombin clotting time (p.c.t.) tests,[37] of which the whole blood ("bedside") method and Quick's plasma prothrombin test are preferred clinically, while the Iowa two-stage method is said to have additional advantages in the securing of research data. None of these tests can meet certain theoretical objections which aim at deciding whether prothrombin, as such, is the only significant variable they measure. When we know more about the thromboplastic and inhibitory factors it is not unlikely that the evaluation of the p.c.t. test will have to be modified. Despite these objections, the prothrombin clotting times are valuable clinically both in diagnosis and treatment. As an example of the latter, we have the use of the hypoprothrombinemic dicumarols[24] in the prevention and symptomatic cure of thromboembolism. In order to produce prothrombin the liver must be supplied with adequate amounts of certain naphthoquinones (vitamins K).[6] Usually these are readily absorbed from the alimentary canal from bacterial as well as food sources, but deficiency may occur, as in hemorrhagic disease of the newborn and some forms of intestinal disease. Vitamin K therapy requires consideration of absorption (e.g., bile salts and fat-soluble menadione, etc., given orally) and of ability of liver utilization even when water-soluble vitamin K is given parenterally. Large doses of vitamin K supplement transfusions in combatting overdosage of dicumarol during anticoagulant therapy.

The physiological controls of phospholipid and thromboplastic enzyme are unexplored. It is known that cephalin is never deficient and that there are variations in plasma or serum enzyme, at least in active tryptase. The inhibitor problem is also unsolved except for data showing antithrombin and heparin increase in peptone and other anaphylactoid shock.

We can modify the coagulability of the blood *in vivo*, particularly in the direction of prolonged clotting times, both by heparin injections and by dicumarol therapy.[30] The former is promptly initiated but hard to maintain, for reasons involving the excretion, destruction (e.g., by heparinase) and other fates of heparin in the body.[1] These topics will be discussed fully in subsequent papers but a preliminary word on the basic mechanisms of action of hypoprothrombinemic agents may be appropriate. Dicumarol or 3,3-methylene-*bis* (4-hydroxy) coumarin was isolated and identified by Link et al.,[24] as the toxic agent responsible for the hemorrhagic disease developed in cattle from eating spoiled sweet clover hay. Quick[30] had shown this disease to be characterized by a severe hypoprothrombinemia. Dicumarol, in common with a number of related drugs, has no significant action on the clotting mechanism proper but merely

serves to depress the liver production of the essential factor, prothrombin. In addition to the hypoprothrombinemia there may also be a lowering of the plasma fibrinogen with excessive doses of the drug. In some ways the liver response to these drugs is just the opposite of that to the vitamins K. It is perhaps interesting, therefore, that phthiocol (a vitamin K naphthoquinone from the tubercle bacillus) can be converted into 3, 3-methylene-*bis* (2-hydroxyl-1, 4-naphthoquinone) which has hypoprothrombinemic properties, and conversely that 3-methyl-4-hydroxycoumarin has some vitamin K activity. Indandione derivatives, chemically related to phthalic acid which antagonizes dicumarol, have the actions not of vitamin K but of the antiprothrombinemic agents. Excess of vitamin K appears to have a liver-stimulating action able to produce hyperprothrombinemia in the normal body as well as to aid in counteracting overdosage by dicumarol. Dicumarol can be synthetized from salicylic acid, and salicylates (especially acetylsalicylic acid) have a weak but quite definite hypoprothrombinemic tendency. A conservative interpretation of all these data emphasizes their close connection with liver cell function and leads to hesitation about postulating any direct chemical antagonisms between the two main types of agents that raise or lower plasma prothrombin, respectively.[15]

It is not yet practical to enhance the general coagulability of the blood by any safe systemic procedures other than fresh blood transfusions and, perhaps, the use of certain plasma globulin fractions in hemophilia.[23] Thrombin, especially aided by fibrinogen solution, fibrin foam, fibrin film, gelfoam and soluble cotton (oxidized cellulose) has a wide variety of modern uses as a local hemostatic and coagulant. Thromboplastic preparations are also restricted to topical use and are in general less effective than the new thrombin materials for this purpose.[14]

Finally, in relation to the specific problem of thromboembolism we must consider the rôle of local conditions, especially damage to endothelium of the vessel wall and stagnation of blood flow, freezing (frost-bite), heating (burns), etc. There is room for new detailed information on the exact way in which these factors contribute to the local disturbance in the clotting mechanism. It would seem reasonable, however, to orient the direction of these inquiries along the lines opened up by consideration of the individual factors in the clotting mechanism, as brought out in the experimental analysis and to which we have devoted major attention in the preparation of this review.

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# Combined Staff Clinics

## Hemolytic Mechanisms

THESE are stenotyped reports of combined staff clinics of the College of Physicians and Surgeons, Columbia University. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Frederick K. Heath.

DR. FRANKLIN M. HANGER: The purpose of today's discussion is to point out some of the factors involved in the breakdown of red blood cells.

It would be quite impossible in the time allotted to enumerate the various hemolytic processes or to present the clinical pictures of the diseases characterized by excessive blood destruction. It is rather humbling to medical science to recognize that despite intensive study by the most refined physicochemical technics there is a great deal lacking in our fundamental knowledge of the structure of the simplest cell of the body, the red blood cell. We know that the erythrocyte springs from the bone marrow as a relatively large flat cell, lithe and elastic, the so-called reticulocyte. As the red cell circulates through miles of capillaries it is constantly subjected to warping and distortion but promptly resumes its discoid shape when the stress is released. Gradually it manifests the conventional ageing process, i.e., "thickening about the middle," and as it becomes more spheroid it loses its elasticity so that threading the small capillaries becomes more hazardous and mechanically difficult. Many red blood cells fragment in the surge of the circulating blood while others find stagnation in the recesses of the spleen more to their liking and there pass to their oblivion by other physiological processes.

The average life of the erythrocyte is about 120 to 125 days. It has been estimated that the normal individual destroys 10,000,000 red cells per second. By such reckoning the carnage transpiring in this auditorium surpasses comprehension.

It would be most helpful in the study of the breakdown of the red cell if more were known regarding its detailed histology. Chemical analysis indicates that about 70 per cent of the cell mass is comprised of water, about 25 per cent is hemoglobin and only about 3.5 per cent represents the stroma.

It is the structure of the stroma which chiefly determines the shape of the erythrocyte. The stroma is composed of lipids such as lecithins, cephalins and sphingomyelins, and of a peculiar protein called "stromatin" which resembles collagen more than any other tissue substance found in the body. Much of this protein is not free but is combined with the lipids. Because of the relatively small amount of stroma in the cell it is necessary to assume the distribution of some of its constituents in organized molecular layers particularly at the surface. It has been calculated by Ponder and others that one protein molecule probably orients a mosaic containing about ninety lipid molecules. The lipids are chiefly arranged in monomolecular palisades, with the fatty acid poles directed centrally and the phosphoric acid-choline portion more or less facing the exterior surface. The proteins are probably arranged tangentially to the surface of the cells and are said to be more concentrated in the area of the disc concavities.

The interior of the erythrocyte is even less well understood. We know from the amount

of hemoglobin in the cell that the molecules must be very closely packed and heavily hydrated. In other words the hemoglobin is probably in the form of a gel. Whether the stroma permeates the cell is not known, but stromatin probably forms a gel readily, so that the most probable structure is one of strands of semiliquid protein material forming a matrix which in turn is conjugated loosely with the hemoglobin mass. That the normal red cell maintains its discoid shape is due not only to the unique organization of the stroma but also to a plasma albumin which is absorbed onto the surface of the cell.

Hemolytic agents operate by disturbing the intricate molecular organization of the stroma. Saponin, for example, when added in very dilute solutions to washed red cells first causes small discreet irregularities to appear on the surface of the erythrocyte, indicating a focal reaction of the hemolytic agent with certain groupings comprising the cell membrane. When the hemolysin is used in stronger concentration increasing numbers of crenation points appear until the entire surface is thickly studded. At the same time the erythrocyte tends to become more spherical and finally is transformed into a swollen, distorted sphere with a smooth surface. During the first phases of this process hemolysis may be prevented by washing off the saponin or by adding normal serum which neutralizes the hemolytic action of this and many other substances. The red cell then tends to return to its original shape. If, however, hemolysins are permitted to act until the red cell becomes a smooth sphere, the process becomes irreversible and the distended erythrocyte vanishes rather suddenly from the microscopic field. At that moment the hemoglobin which is confined in some kind of orderly arrangement within the cell rapidly flows out through the open pores and only a gossamer-like unit of stroma ("ghost") remains of the original structure. It is probable that most hemolytic agents, whether they be a saponin or a lipid solvent or a specialized protein fixed to the cell, disrupt the erythrocyte by this same mechanism of disorganization of the surface structures.

DR. SIDNEY C. WERNER: Can the hemoglobin leave the red blood cell without hemolysis in normal circumstances?

DR. HANGER: No, that is hemolysis. The clinical picture of hemolytic diseases is determined to some extent by the location within the body where red cell breakdown takes place. Hemoglobinemia is more prone to occur when the process is intravascular. On the other hand, when red cell destruction takes place within the spleen or bone marrow the liberated hemoglobin is taken up immediately by the contiguous cells and is transformed to bilirubin by intracellular processes. In these cases an acholuric jaundice rather than hemoglobinemia and hemoglobinuria is the rule. Normally 2 or 3 mg. per cent of hemoglobin is found in plasma due to mechanical fragmentation of red cells which is constantly taking place in the circulating blood. Under pathological conditions hemolysis may be so severe that three-quarters or more of the red cells of the body are destroyed within a few hours and large amounts of hemoglobin, exceeding 300 to 400 mg. per cent may appear in the plasma. In such cases a portion of the hemoglobin is excreted in the urine; another portion is taken up by the reticulo-endothelial system to be converted to bilirubin and some is transformed to hematin which at once combines with albumin to form "methemalbumin." Methemalbumin, a substance with a characteristic absorption spectrum recently described by Fairley, is relatively persistent in the blood stream since it is not excreted by the kidneys and is slowly absorbed by the reticulo-endothelial system.

When free hemoglobin in the plasma exceeds 135 mg. per cent it tends to appear in the urine and after levels exceeding 200 mg. per cent are reached, the amount of hemoglobin excreted by the kidneys is proportional to the blood level. In the lower ranges tubular re-absorption has been shown to play a rather important rôle. Hemoglobin, which has approximately the molecular weight of albumin, passes through the glomerulus about 3 per cent as readily as creatinine. When it occurs in the glomerular filtrate in amounts less than 2 to 3 mg. per cent none appears in the urine; above these levels, tubular reabsorption becomes inadequate and hemoglobinuria develops. If the tubules become injured by repeated exposures to hemoglobin and its derivatives, the reabsorptive power may become impaired; hence, in chronic hemolytic diseases hemoglobinuria may be observed when the blood levels are lower than 130 mg. per cent. Also, in the condition known as "march hemoglobinuria" a disturbance of tubular reabsorption must be assumed. Patients with this rare condition are usually young male adults who develop red or brownish urine after prolonged walking and running. The amount of blood destroyed during the episode has been shown to be negligible and except for hemoglobinuria symptoms are few. The condition is usually a temporary one and is of little clinical importance. Posture has been assumed to play a rôle in the red cell breakdown and in altering renal function but the disturbance has not been adequately explained.

We might turn now to the formation of bile pigments from hemoglobin. Dr. Stetten will review briefly for you the breakdown of hemoglobin and will trace the various derivatives through the reticulo-endothelial system, blood, liver and intestinal tract.

Dr. DeWitt Stetten, Jr.: At the outset, I should like to call your attention to a recent review by Watson,* which contains a critical discussion of many of the points to be covered in this clinic.

In addition to hemoglobin there are several biological pigments of the iron-porphyrin-protein type, among others catalase, some of the cytochromes and myoglobin. Other than the fact that myoglobin has been shown to contribute to the bile pigments, little is known of the catabolism of these compounds, but it may be supposed that the iron-porphyrin portion of these molecules is handled by the body in a fashion similar to that of hemoglobin.

The normal destruction of the vast majority of red cells occurs not in the free circulation but, as Dr. Hanger has indicated, in the reticulo-endothelial system. Of the three major portions of the hemoglobin molecule, we may speak with some assurance of the disposition of the protoporphyrin and of the iron, but we have very little information as to the intimate fate of the globin which in terms of weight is the major portion. The porphyrin nucleus, it will be recalled, comprises four five-membered rings of the pyrrol type bound to each other by carbon bridges, called methene bridges, of which the one designated $\alpha$ (Fig. 1) is of importance to the present discussion. About the periphery of the nucleus are several substituents, in the case of protoporphyrin, vinyl, methyl and propionic acid. The point of attachment to globin is according to some authorities over these propionic acid side-chains, while the iron always of the divalent or ferrous variety in normal hemoglobin is bound to the pyrrol nitrogen atoms, two by primary valence and two by coordinate valence. Whereas the porphyrins in general are extraordinarily resistant to oxidative ring rupture, the presence of an atom of iron in the center of the nucleus alters this stability and renders the ring system susceptible

* Watson, C. J. Some newer concepts of natural derivatives of hemoglobin. *Blood, J. Hematol.*, 1: 99, 1946.

to oxidation even by such mild agents as $H_2O_2$. This is the first step in the biological destruction of hemoglobin, the oxidation of the porphyrin at the $\alpha$-methene group but without the elimination of either the iron or the globin. The green pigment which results has recently been called verdohemoglobin; it is normally present in red cells to a very appreciable extent and is familiar to all of you as the material formed from hemoglobin by Streptococcus viridans. It is characterized by the ease with which on treatment with dilute acid it may lose its iron, in contrast to hemoglobin in which the iron is more stably held. In fact, so easily does it lose its iron that this inevitably happens and we are left with biliverdin-globin.

At this point it will be well to review briefly the fate of the iron. You will remember that the body has no efficient method for getting rid of iron, that the iron lost per day except for such accidents as hemorrhage or hemoglobinuria is trivial. The quantity of iron contained in the body is determined not by the rate of its excretion but by the rate of its absorption across the intestinal mucosa. This, in the normal animal, is very small but for reasons not well understood becomes vastly larger after hemorrhage. Since iron cannot be eliminated from the body it follows that the iron released by verdohemoglobin must be efficiently stored and re-utilized. Today the nature of this storage is fairly well understood. A curious protein, ferritin, occurs in the spleen and liver into the interstices of the lattice of which tremendous quantities of hydrated ferric oxide may enter without detectable alteration in the dimensions of the lattice. In fact, so much ferric oxide may enter this molecule as to bring its iron content close to 25 per cent; not only may it enter readily but it may leave just as easily. This reversible introduction of iron into ferritin, first demonstrated to occur *in vitro*, has recently been shown to occur in the living animal. Hepatic and

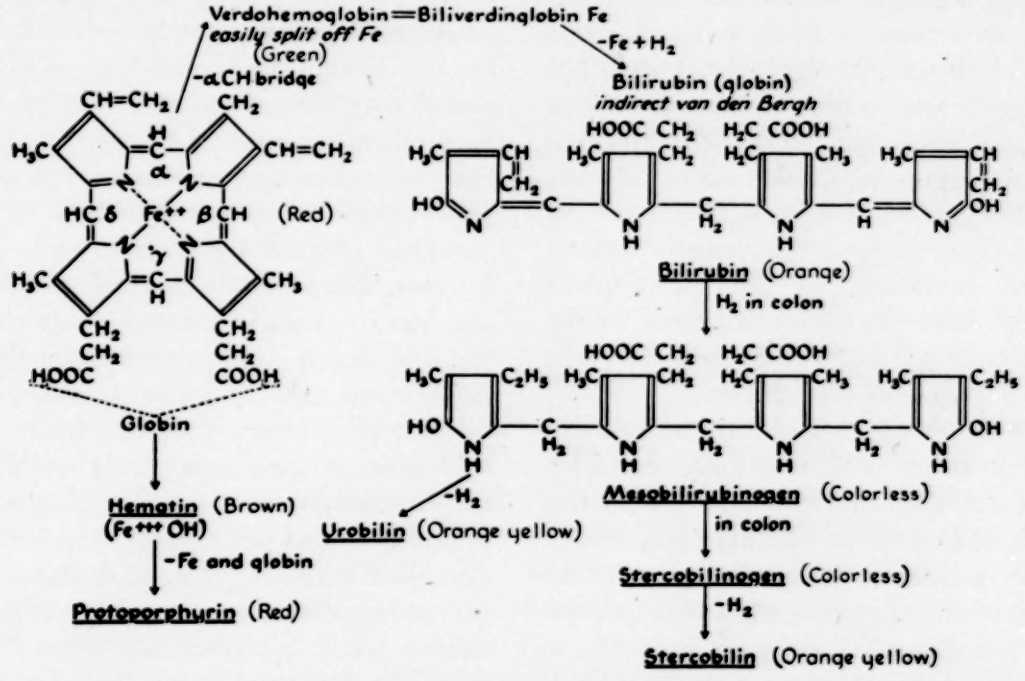


FIG. 1. Some of the more important natural derivatives of hemoglobin. (From WATSON, C. J. Some newer concepts of the natural derivatives of hemoglobin. *Blood, J. Hematol.*, 1: 99, 1946.)

splenic ferritin, therefore, may be regarded as a warehouse for the transient storage of iron arising from the breakdown of verdohemoglobin and awaiting re-incorporation into fresh hemoglobin.

To return to the biliverdin, this is now reduced to bilirubin and for the biochemist it is impossible anatomically to localize this reaction. Practically every tissue that has been investigated seems to be capable of bringing it about and a wide variety of naturally occurring reductants, mostly breakdown products of glucose, serve as reagents. There is some reason to suspect that this reduction in the mammal is associated with glycolytic processes in the liver in that it apparently fails to occur when the liver is depleted of glycogen.

Bilirubin is a normal plasma constituent in concentrations of 0.25 to 1 mg. per cent. It occurs in two modifications which are currently called hemobilirubin and cholebilirubin. Hemobilirubin, which makes up the bulk of the normally occurring plasma pigment, is supposed to be the initial product and according to some authorities is still firmly linked to the original globin residue. It is non-dialyzable, does not cross the renal glomerulus and gives an indirect van den Bergh reaction. It is suggested that only in the liver is the pigment dissociated from its protein but whether by the Kupffer cells or the polygonal cells is not clear. The free pigment, as its ion, is normally injected into the bile. Should there be an obstruction to the bile flow, the bilirubin is regurgitated into the blood stream supposedly through the cells of the ampulla of the bile capillary. Once in contact with plasma proteins this regurgitated bilirubin now associates itself loosely with serum albumin with which it migrates. This, cholebilirubin, is characterized by dialyzing readily across both artificial and glomerular membranes and by the direct immediate van den Bergh reaction. Whereas the indirect reacting pigment does not appear in the urine even when present in the blood in high concentration, cholebilirubin has a low renal threshold of 1 to 2 mg. per cent and appears regularly in the urine when this concentration in the blood is exceeded.

In the intestinal tract, probably because of the reducing action of the intestinal flora, bilirubin undergoes a series of hydrogenations which give a mixture of products, mesobilirubinogen and stercobilinogen; this mixture according to Watson is best referred to as urobilinogen. Urobilinogen is in part absorbed into the portal circulation but the normal liver effectively removes about 98 per cent from the blood and re-injects it into the bile. Some 2 per cent escapes the liver, travels into the systemic circulation and ultimately is excreted in the urine. Should liver function be impaired, the complement of urobilinogen escaping the liver and appearing in the urine may be expected to rise. The complete lack of urobilinogen in the urine is rarely seen except under conditions when bilirubin has been excluded from the intestinal tract. The last step in this reaction sequence is not a biological reaction at all but an autoxidation of urobilinogen, whether urinary or fecal, to give urobilin. This pigment like the foregoing turns out to be a mixture of two components, urobilin 9 $\alpha$ and stercobilin. The only importance of this reaction is the fact that the products do not give a color with Ehrlich's aldehyde reagent, the conventional test for urobilinogen, and therefore this test should be performed upon fresh specimens.

DR. HANGER: The reactions outlined by Dr. Stetten are of clinical and diagnostic importance. In hemolytic syndromes the amount of stool urobilin (stercobilin) may be enormously increased. The normal output of this substance in the stool in twenty-four hours rarely exceeds 200 mg. but in hemolytic jaundice this amount may be ex-

ceeded ten-fold. The stool urobilin excretion may be employed as a measure of blood breakdown. In anemic patients, however, absolute values may be misleading. For example, the excretion of 200 mg. of stercobilin in twenty-four hours by a person with a red blood cell count of 1,000,000 would be abnormally high and an indication of excessive blood cell breakdown in that individual. Another factor leading to erroneous interpretation is that in cases of violent hemolysis there frequently is considerable injury to the liver. Under these conditions, shock with focal necrosis of the liver is not uncommon and the jaundice that develops may be due in part to hepatic injury, with a direct van den Bergh reaction in the serum and bile appearing in the urine.

TABLE 1

FACTORS LEADING TO RED BLOOD CELL DESTRUCTION

1. Mechanical fragmentation
2. Spherocytosis
3. Fixation of specific globulins to red cell surfaces
4. Stasis
5. Splenic activity
6. Injury of cell by exogenous chemicals
7. Metabolic hemolysins

We will now consider some of the more common factors which promote the destruction of red cells. (Table 1.) Reference has already been made to mechanical fragmentation which is probably the chief cause of red cell breakdown in the normal individual and to the development of spherocytosis which usually antecedes hemolysis.

Hemolysis may also be caused by certain globulins which may become attached to specific structures on the red cell surface. Some globulins are primarily injurious to the cell causing direct disruption of the structure; others "sensitize" by becoming fixed to the cell ("amboceptor") but the presence of "complement" is requisite for the occurrence of hemolysis. Even the attachment of amboceptor is said to increase the fragility of the sensitized red cells to mechanical stress and strain.

Other serum globulins are not directly hemolytic but are injurious to red cells by inducing agglutination (agglutinins). Clumping of erythrocytes causes them to rupture more readily in a shaking apparatus and presumably accelerates breakdown in the circulating blood.

Stasis of blood in the spleen is regarded by many as a factor leading to destruction of red blood cells in health and in disease. It has been pointed out that spherical cells and clumped cells especially tend to be enmeshed in the intricate vascular bed of that organ, where actice phagocytosis of red blood cells can be demonstrated to take place. Studies by Dameshek and others suggest that in certain diseases the spleen may promote spherocytosis and increased red cell fragility. It is probable that actual contact of the red cell with the cells of the sinusoids rather than a secretion by the spleen is necessary for this action. Stasis, therefore, may be an important preliminary stage in the aging and elimination of the erythrocyte.

The chemical agents with hemolytic properties are very numerous and varied and will not be specifically enumerated here. Many of these substances which are highly lytic to washed red cells *in vitro* exert but little effect in the living organism because of the inhibiting action of serum. Conversely, a number of drugs such as sulfonamides and plasmochin (notably in the Negro race) which have but little intrinsic hemolytic activity may cause severe hemolytic anemia in particular cases. Some agents, such as phenylhydrazin and certain snake venoms, show hemolytic properties with predictable certainty but a large group produce symptoms only in persons with natural or acquired idiosyncrasies to that particular chemical. Favism is a form of severe hemolytic anemia common in certain Mediterranean regions and is caused by contact with the fava bean or vine. The disease

occurs primarily in those who manifest a sensitivity to certain products of the plant. The mechanism by which hemolysis is caused by drugs, bacterial toxins etc., in the body is not known but may involve injurious conjugation of the hemolytic agent or its derivative with the patient's cells, or the excitation within the organism of hemolytic processes which normally are held latent. The tissues themselves contain many products of metabolism which are potentially hemolytic, such as fatty acids, bile salts, soaps, lecithins and lysolecithins. The question is often raised whether under special conditions these naturally occurring substances may not be instrumental in breakdown of red cells. The lysolecithin theory is an attractive one, and I have asked Dr. West to discuss it for us.

DR. RANDOLPH WEST: The literature on lysolecithins was summarized to 1941 by Singer* and I refer you to his article on the subject. The first information was gained indirectly through study of cobra venom and allied snake venoms. The earlier work was done in 1860 by Weir Mitchell. In 1902, Flexner and Noguchi studied the phenomenon carefully and found that cobra venom would not bring about hemolysis of washed red blood cells and that the presence of serum was necessary for hemolysis. They then determined that cobra venom contained an enzyme which when acting on lecithin present in serum produced a form of lecithin which was lytic. From a study of snake venoms it was found that an enzyme and lecithins of serum produced a lytic lecithin which acted on red cells.

The problem was then carried on by Fahraeus who noted a difference in the sedimentation rate of blood from the splenic artery and from the splenic vein. The sedimentation rate was more rapid in the splenic artery than in the splenic vein because lysolecithin inhibits rouleau formation.

Further studies were carried out defining the conditions under which lysolecithin was formed in serum. In summary, they are as follows: If blood be drawn and placed immediately in the icebox so that serum is separated at low temperatures, then that serum, if left to stand without stirring, will give rise on subsequent incubation at body temperature to a lytic form of lecithin which can be extracted by organic solvents and tested for hemolysis against washed red blood cells. That is the general technic for determining the lysolecithin content of serum.

Stirring decreased the amount of lysolecithin formed. An analogy exists with stagnant and circulating blood. Under conditions of complete stasis the action of the enzyme, which has many properties in common with complement, is much greater than when the blood is agitated.

This led to the theory that stagnant blood anywhere in the body, and particularly in the spleen, gives rise to an increased rate of production of lysolecithin which then might be a factor in hemolysis of red cells in the body. Studies reported on splenic artery and splenic vein blood and on blood from varicose veins, show a considerable increase in lysolecithin in stagnant blood over blood that is circulating rapidly in the general circulation. Lysolecithin, however, has not been demonstrated to act in the presence of plasma or serum and acts only on washed red blood cells, so its position as an active factor in bringing about hemolysis in disease of man is still open for further investigation.

DR. HANGER: Hemolysis in disease may depend upon one or more of the factors that have been discussed. In Table II these conditions have been arbitrarily separated into (1) those in which a demonstrable defect of the red cells may be significant, (2) those in

* SINGER, K. Lysolecithin and hemolytic anemia. The significance of lysolecithin production in the differentiation of circulating and stagnant blood. *J. Clin. Investigation*, 20: 153, 1941.

which a serological factor is either demonstrable or suspected and (3) those in which abnormal splenic activity (hypersplenism) is present.

In general, the symptomatology of the hemolytic state depends on the rapidity

TABLE II
HEMOLYSIS IN DISEASE

A. Red Blood Cell Factors:
   1. Anoxia susceptibility
   2. Congenital spherocytosis
   3. Acid susceptibility
   4. Heat injury
   5. Malnutrition
   6. Idiosyncrasies to certain chemicals and drugs
   7. Parasitic infestations and bacterial infections
B. Serological Factors:
   1. Naturally occurring agglutinins
   2. Specific antibodies to red blood cell agglutinogens
   3. Heterologous antibodies to red blood cells
   4. Cold agglutinins (reversible)
C. Splenic Factors:
   1. "Hypersplenism"

with which blood is destroyed and whether the destruction takes place within an organ rich in reticulo-endothelial elements or free in the vascular system. Patients during brisk hemolysis often develop headache, extreme backache, abdominal pain and pains in the legs. There is frequently a chill followed by a rapid rise of fever; there may be vomiting and mental disturbances. The spleen often enlarges and may be tender. Within a few minutes or hours it will be noted that the urine is bright red or dark brown. In severe cases anuria develops rather quickly and there are often manifestations of shock, air hunger and signs of acute anemia. Rapid hemolysis is always a serious affair. One of the current misconceptions is that the anuria which develops is due primarily to the precipitation of hemoglobin by acid urine in the renal tubules and that alkali administration should be vigorously maintained until the urine becomes alkaline. At autopsy in these cases it is quite true that hemoglobin casts are often found in the tubules but recent investigations tend to indicate that crystalline hemoglobin is not toxic and is not precipitated by acid urine. It is more probable that anuria in these cases is due to shock, secondary to decreased renal circulation. Hemoglobin casts are found in the tubules only when hemoglobin is administered after anuria has developed and are dependent upon, rather than the cause of, the urinary shutdown. It is true in animals with acidosis that the hemoglobin tends to break down into acid hematin and to methemoglobin which may be injurious to renal tubules but the necessity for alkalinizing the urine is probably not as important as is generally taught. Moderate doses of alkali are not contraindicated but excessive doses may be toxic and the alkalinity of the urine should not be used as the sole index of adequate therapy. The most important treatment of massive hemolysis is the prevention of shock and this is usually best attained by liberal parenteral administration of fluids, preferably transfusions. Some authorities raise the theoretical objection that the patient is destroying a great deal of blood and to give transfusions merely supplies more fuel for the flame. This objection is probably more theoretical than real and I repeat the important treatment of the hemolytic crisis is to maintain adequate blood pressure and adequate renal blood flow if possible.

DR. YALE KNEELAND, JR: I should like to pin you down specifically. If you see a case of black water fever with massive hemolysis, you do not believe it is necessary to give intravenous alkali. Is that correct?

DR. HANGER: According to the best information you do not poison your patient with alkali. You alkalinize mildly. It apparently is not the crucial thing. Recognizing that these anemias are violent and acute, I should say that transfusion and the supportive measures are probably the main factors.

DR. JOHN DEAN: Why should one alkalinize at all?

DR. HANGER: Chiefly on the basis of the observation that when acidosis occurs there tends to be formation of methemoglobin which apparently affects tubular function. It also diminishes the blood flow through the kidney.

DR. ROBERT F. LOEB: I do not believe that the British group who have studied black water fever will subscribe. I believe it is customary in black water fever to give massive doses of bicarbonate in one way or another. I think there is evidence to indicate that would make a difference.

DR. HANGER: To come back to Dr. Kneeland's question, I would give alkali but I would not regard it as the most important part of the therapy.

Among the hemolytic disorders in which a concomitant defect in the red cells is demonstrable is sickle-cell anemia. I have asked Dr. Turner to give us a brief survey of this problem.

DR. JOSEPH C. TURNER: In 1910, sickle-cell anemia was first described in this country by Herrick and it has been recognized since that time as an important type of hemolytic anemia in any community having a large number of negroes. The name itself carries perhaps the unjustifiable implication that the property of sickling is a sufficient explanation for the disease. While it is probably true that sickling is an important factor in pathogenesis, we do not know precisely how sickling is involved in the hemolytic process and what its relation may be to the various other pathological manifestations.

We may begin by considering the so-called sickling "trait." This, as you undoubtedly know, is found in the colored race all over the world, in Africa, North America and Central and South America in from 5 to 20 per cent of the population. It is inherited apparently as a dominant Mendelian character and the vast majority of people who bear it are healthy. Only in perhaps 1 or 2 per cent does sickle-cell anemia develop. The anemia usually occurs early in life although we have seen patients in this clinic with the first manifestation of sickle-cell disease at the age of twenty-five or thirty. Death before middle age is the rule. Sickle-cell anemia has been described in the white race. Usually the people involved are Mediterraneans, Greeks, Sicilians or Arabs and one may suspect that at some time in the past there has been an admixture of negro blood. Such cases are extremely rare.

Sickling may be defined as a reversible distortion of the erythrocyte which occurs under lowered oxygen tension. The method ordinarily employed to demonstrate sickling is quite simple and I have no doubt you are all familiar with it. A drop of blood is placed on a slide, covered with a slip, sealed with paraffin and then placed in the incubator. The slide is examined microscopically at intervals of three hours or so.

The course of sickle-cell disease is extremely variable and may run from one to fifteen years. During this time a great variety of clinical manifestations may be encountered. One of the most curious is the leg ulcer, usually found around or just above the ankle. These ulcers are persistent and extremely difficult to treat. There may be rheumatic manifestations which simulate acute rheumatic fever almost precisely. Pains occur in and about a variety of joints and, moreover, one may find cardiac enlargement as well as systolic and diastolic or presystolic murmurs at the apex. Any negro suspected of having rheumatic fever should have his blood examined for the presence of sickling.

No less dramatic than these manifestations of the disease are those which are thought to be associated with changes in the small blood vessels in the shape of vascular thrombosis or circulatory stasis. One finds for instance neurological disturb-

ances. Since any area of the brain may be affected the symptoms vary from mere drowsiness to a fatal cerebral accident. In similar fashion bones may be involved. Areas of infarction occur which may be responsible for acute pain. These, if they involve the spine, may take the form of the common syndrome of lower back pain. We have seen a man here who entered with such a complaint and on *x*-ray examination he had an area of bone destruction in a lumbar vertebra. Such findings are presumably related to obliteration of nutrient arteries.

The most critical aspect of sickle-cell disease is the so-called abdominal crisis, which so frequently ushers in a fatal phase of the disease. Severe abdominal pain is usually accompanied by some muscular rigidity, in consequence, the clinical situation closely resembles a surgical condition of the abdomen and differential diagnosis may be extremely difficult. In sickle-cell disease such an abdominal crisis may be followed by a rapid destruction of blood with death in a matter of days or even hours. Shock is usually a significant part of hemolytic crises and must be treated vigorously.

So much for a brief view of the outstanding clinical manifestations. As for the pathological condition it is both curious and rather unsatisfactory. In children the spleen has been found enlarged but in adults this organ has frequently been almost destroyed—probably a manifestation of atrophy secondary to vascular thrombosis. The spleen may weigh only 10 or 15 Gm. Splenectomy, I should say, has not proved to be of any value in treatment.

The most interesting changes, interesting because they seem to be related to many of the clinical manifestations, are in the small blood vessels. These may be everywhere engorged and virtually occluded by what appear to be clumps of agglutinated sickle cells. They may, moreover, show an actual anatomical change in the vessel wall, as thickening, intimal proliferation or tortuosity. All such changes could act to promote circulatory stasis and the physiological equivalent of thrombosis. An organized thrombus is not frequently seen pathologically. The findings, however, fail to account satisfactorily for what has happened in life and pathologists have sometimes been forced to conclude on examination only that the patient died of anemia.

We come now to the sickle cell itself, the phenomenon of sickling and its physical and chemical analysis. Sickling appears to be a property of the cell and not dependent upon the presence of serum. That is to say, if the cells are washed they still sickle. This does not imply, however, that there may not be substances in the serum which affect sickling. Indeed, there are substances of a non-specific sort that can influence sickling, such as bile salts and pH. If a preparation of washed cells is made in a chamber which is designed to permit evacuation of air and flushing of the system with gases, one finds that on removal of air and the substitution of an oxygen-free gas such as nitrogen, carbon dioxide or ethylene, sickling occurs in a few minutes. Then if, air or oxygen is re-admitted the cells spring back into normal shape instantaneously. Carbon monoxide is also capable of reversing the process of sickling and so it has been attractive to think that the process is associated with reduction of hemoglobin; it may be but the proof is wanting. We have examined hemoglobin prepared from sickle cells spectrophotometrically and can find in the absorption curves no abnormality.

It has been shown that the mechanical fragility of sickle cells is increased, but as yet we have no complete or satisfactory account of the relation of sickling to an increased rate of hemolysis.

As for treatment there is little to say. Oxygen has been employed over a period of one week to four weeks in an attempt to

alleviate the symptoms in sickle-cell crises. It appears that the number of sickle cells in venous blood is reduced as the result of such therapy but the process of hemolysis seems to be unaffected by the treatment. Transfusion has been employed, not only for the shock which occurs in the acute crisis, but also in an attempt to hasten healing of the leg ulcers. It is difficult to say just how much transfusion will accomplish in such cases but our impression is that it is sometimes of benefit.

TABLE III

| | Splenectomies | Died following Operation | Died of Disease | Died of Other Causes | Total Deaths |
|---|---|---|---|---|---|
| Congenital hemolytic icterus | 56 | 3 | 1 | 5 | 9 |
| Idiopathic purpura | 58 | 0 | 4 | 5 | 9 |
| Banti's | 96 | 11 | 28 | 4 | 43 |

DR. HANGER: Another disease characterized by hemolytic anemia and abnormalities of the red blood cells is congenital spherocytosis. This is as much a surgical problem as a medical one and I have asked Dr. Elliott to discuss certain aspects of this disorder.

DR. R. H. E. ELLIOT, JR.: Congenital hemolytic icterus, or as it is more properly called, spherocytic jaundice can in most instances be completely arrested by removal of the spleen. It is in this particular blood dyscrasia that splenectomy has yielded its most brilliant results. The disease as will be remembered is characterized by jaundice, weakness, anemia and an enlarged spleen. The onset usually dates back to childhood and in most cases but not in all a familial history is obtainable. The characteristic blood findings in this disease are the presence of anemia, the presence of spherical microcytes, a reticulocytosis and increased fragility of the red cells.

Table III emphasizes the fact that in three groups of splenopathies in which splenectomy was performed in this clinic the results have proved to be the most satisfactory in spherocytic jaundice. As will be noted, only one patient died of a recurrence of the disease as opposed to four in purpura. It is also worth calling attention to the fact that there were three deaths following operation. This is mentioned to stress the fact that the operation is obviously not without its hazards in certain instances.

In addition to the presence of jaundice and the various other clinical and laboratory findings previously described gallstones are also found in from 40 to 60 per cent of all cases. This is understandable when it is remembered that there is a tremendous amount of red cell destruction going on, particularly in the spleen, and that the pigment so released is carried directly to the liver where the increased excretion of the pigment in the bile may lead to the formation of stones. These stones are notably soft and in some instances can be crushed at the time of operation.

The results of splenectomy in atypical hemolytic icterus have been disappointing. We have collected information on eleven patients who have undergone this operation and five of these eleven are now dead of the disease. This does not necessarily mean, however, that splenectomy is contraindicated in this group. It has become the feeling in the Spleen Clinic that individuals who have an undiagnosed hemolytic anemia with a large spleen are entitled to splenectomy, provided examination of the bone marrow reveals no contraindication. Furthermore, it is believed that the presence of the spherical microcyte is in most instances necessary to the prediction of a satisfactory outcome after splenectomy. In other words, when the spherical microcyte is present the result will usually be favorable.

The one patient indicated in Table III as having succumbed to a recurrence of spherocytic jaundice after the spleen had been removed is worthy of mention. Similar

instances of recurrence have been reported in the literature. This particular patient was autopsied and the entire left upper quadrant of the abdomen found to be the site of multiple small nodules of accessory splenic tissue. Whether these were the result of the spillage of splenic tissue during operation or whether some accessory spleens had been left behind at operation is not known but both of these mechanisms have been suggested in the literature and do apparently occasionally occur.

It seems fitting at this juncture to mention briefly the experimental approach* used in this clinic a number of years ago in an attempt to elucidate the rôle of the spleen in this disease.

In 1936, Knisely,† who was studying the anatomy of the spleen, and in particular its circulation, devised the technic of examining the living mammalian spleen depicted in

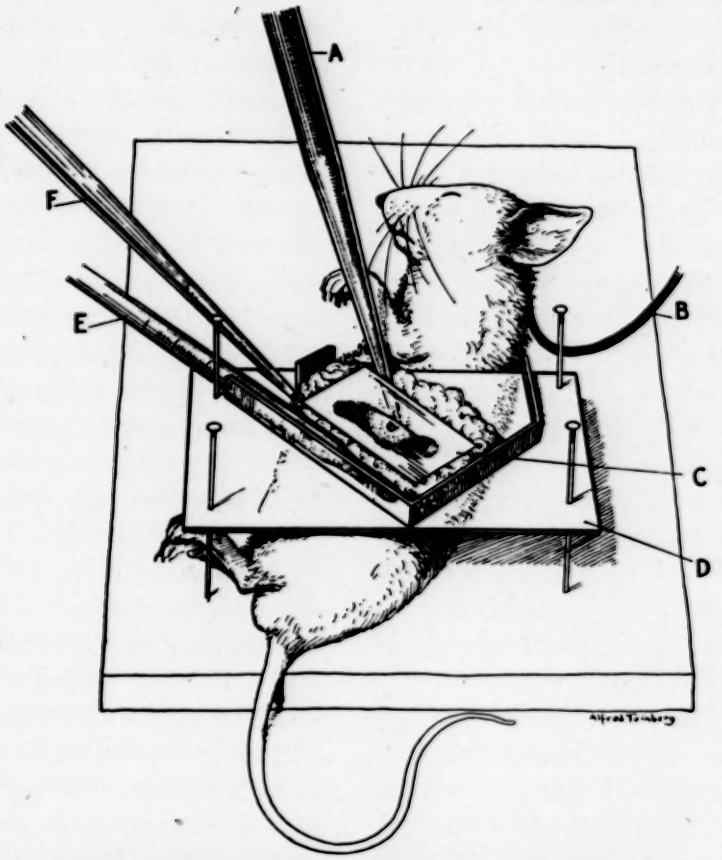


FIG. 2. Diagram of spleen chamber, as applied to the mouse. A, hollowtipped fused quartz illuminating rod; B, anesthesia tubing; C, cover slip roofing spleen chamber; D, celluloid table supporting spleen chamber; E, thermometer; and F, immersion fluid delivery tube. (From MACKENZIE, DAVID W., JR., WHIPPLE, ALLEN O. and WINTERSTEINER, MARGARET P. Studies on the microscopic anatomy and physiology of living transilluminated mammalian spleens. *Am. J. Anat.*, 68: 397, 1941.)

* MACKENZIE, D. W., JR., WHIPPLE, A. O. and WINTERSTEINER, M. P. Studies on the microscopic anatomy and physiology of living transilluminated mammalian spleens. *Am. J. Anat.*, 68: 397, 1941.

† KNISELY, M. H. Method of illuminating living structures for microscopic study. *Anat. Rec.*, 64: 499, 1936.

Figure 11. As can be seen the experimental animal, a mouse, has had its spleen exteriorized. Sodium amytal was used as the anesthetic agent. A fused quartz rod transmits a powerful beam of light through the thin edge of the organ which is then examined with a dissecting microscope brought down over a protective coverslip. Warmed Ringer's solution is used to prevent the spleen from drying out and to maintain a constant temperature.

This approach seemed to lend itself to a study of spherocytosis, a condition which is readily produced in the experimental animal by the use of a hemolytic serum. This serum was produced by injecting the washed red cells of a mouse into a rabbit. Subsequently, the anti-mouse red cell serum so formed was injected into the mouse's peritoneum. This produced spherical microcytes in profusion in the experimental animal and also induced enlargement of the spleen. When these spleens were examined microscopically they were found to bear a striking resemblance to the spleens of humans with hemolytic jaundice.

With the aid of the dissecting microscope it could be seen that the spherical microcytes so formed were unable to pass out of the pulp spaces of the spleen into the collecting veins and venous sinuses. They seemed to be almost selectively held back and did not have the elasticity that the normal erythrocyte had, which Dr. Hanger has previously mentioned. This would seem to explain why we find in the human more spherical microcytes in the pulp spaces of the spleen than in the splenic vein. It would also seem to explain, at least in part, the enlargement of the spleen in this disease and the reason why, after splenectomy, the spherical microcytes and therefore increased fragility persist throughout life despite the disappearance of the jaundice and other clinical manifestations of the disease.

Dr. Hanger: Hemolysis may develop under other clinical conditions in which an abnormality of the patient's red cells is demonstrable. A rare example is paroxysmal nocturnal hemoglobinuria (Marchiafava syndrome) in which the subject manifests hemoglobinuria after sleep but is relatively free of this symptom during the waking hours. Anemia is usually present and dysfunction may develop from the massive accumulation of hemosiderin in the epithelial cells of the kidney. The disease may run a progressively downhill course with increasing anemia terminating often with infection or with vascular thrombosis but may continue as a benign disorder for many years with unexplained remissions and relapses. Ham has demonstrated that the red cells in this syndrome tend to be lysed by normal plasma as well as by the plasma of the patient when the pH is lowered to 6.8. This effect can be attained by bubbling $CO_2$ through a tube of plasma containing the patient's cells. The cause of the symptomatology in man is not clear since the circulating blood probably does not attain this degree of acidity in the living body; furthermore, a thermolabile constituent of human serum is requisite for the phenomenon since hemolysis fails to take place when the patient's red cells are added to acidified heated serum. There is no cure for nocturnal hemoglobinuria but the symptoms are sometimes temporarily ameliorated by the administration of alkalis.

The hemoglobinuria and jaundice that follow heat injury may also be attributed to an alteration of red cells. In severely burned subjects a certain number of erythrocytes become crenated and spherical due directly to the thermal injury. These cells tend to disintegrate quickly within the body.

In malnutrition and deficiency diseases increased fragility of red cells is not demonstrable by ordinary methods. Rhoads has found that erythrocytes obtained from dogs with black tongue are hemolyzed more

readily than normally by certain products of protein metabolism (indol), but it is not yet established that dietary factors play a similar rôle in any of the hemolytic anemia syndromes in man.

Certain micro-organisms, notably the plasmodia of malaria, may cause disruption of parasitized red blood cells. The destruction of erythrocytes in this disease is seldom of the abruptness or the magnitude to produce hemoglobinuria. Black water fever is a rare complication of chronic estivo-autumnal malaria treated with quinine, or rarely, atebrine. It is a grave hemolytic disease more related to the hemolytic idiosyncrasies described above than to physical destruction of the red cells by the parasites. The hypothesis that serum at the time of the hemolytic crisis loses anti-hemolytic properties has not been substantiated. Bartonella infestation is also characterized by hemolytic anemia. Carrion's disease in humans is limited to a small region in the Andes but the recent studies of Pappenheimer on iron-containing bodies resembling bartonella found in the red cells of certain obscure anemias raises the interesting possibility that this type of infection may be more widely disseminated than is generally supposed.

The hemolytic action of certain serum constituents has already been mentioned. It is well known that in human bloods there are naturally occurring agglutinins A and B, upon which conventional blood grouping depends. These isohemagglutinins are found chiefly in the III–1 fraction of Cohn and may be concentrated from this component for diagnostic use. There are also rarer isoagglutinins and naturally occurring hemolysins, such as anti-M, anti-N, anti-P and anti-Rh factors, which are being intensively studied and amplified at the present time. Untoward effects such as capillary embolism and intravascular hemolysis tend to follow incompatible transfusions, especially when the recipient's serum contains agglutinins and hemolysins for the donor's cells. Constant vigilance in cross-matching of bloods must be maintained to avoid transfusion reactions and it must be borne in mind that occasionally hemolysins are present when agglutinins are absent.

Agglutinins and hemolysins may be present in the blood as true antibodies which develop following the injection of red cells containing a certain agglutinogen. Complement is requisite for the demonstration of this type of hemolysis. Anti-$A_2$, anti-M, anti-N and anti-P may develop in human recipients receiving transfusions of cells with the appropriate antigen (agglutinogen). More recently the Rh antigen ($Rh_0$ and its sub-groups $Rh_1$ and $Rh_2$) have been recognized as potential immunizing agents and a sensitizing hazard to Rh negative recipients of Rh positive transfusions or to Rh negative mothers bearing Rh positive children. Erythroblastosis fetalis takes place when the serum of the mother containing anti-Rh factors passes the placental barrier in sufficient amounts to agglutinate and hemolyze the Rh positive cells of the fetus. Studies of the Rh factor lead to intricate biological concepts but from a practical aspect have already advanced fundamental knowledge of natural and induced hemolytic reactions as well as instigating life saving revisions of transfusion technics.

The origin of autohemolysins which Dameshek and earlier French workers have described in the blood of certain cases of acute spontaneous hemolytic anemia is obscure. Theoretically in the presence of certain conditioning substances ("Schleppers") a subject might immunize himself to some antigenic constituent of his own red blood cells and many authorities attribute the phenomenon of black water fever in chronic malaria to this mechanism. It is also possible in other instances that an individual

be sensitized to a complex antigen, such as the Treponema pallidum which fortuitously might contain a sensitizing grouping similar to one occurring in red blood cells. Paroxysmal (cold) hemoglobinuria could be explained by such an assumption. This disorder occurs usually in congenital syphilitics and is characterized by the appearance of hemoglobinuria when the affected individual is subjected to chilling. An autohemolysin (immune globulin) is demonstrable in the serum of the patient which becomes fixed to the red cells only at relatively low temperatures but requires the presence of complement to effect complete hemolysis (Donath-Landsteiner test). In this disease cold is the precipitating factor for the immune reaction, but in most instances of acute hemolytic anemia the process of activation of the autohemolysis is not demonstrable. It is recognized by immunologists that specific antibodies of various types, such as typhoid agglutinins, gradually disappear from the circulating blood after infection but may re-appear after a variety of non-specific stimuli or intercurrent diseases. In a like manner latent specific hemolysin may be liberated from the tissues. Such a mechanism might be explained by the appearance of autohemolysins in the blood following intercurrent infections, allergic reactions, drug sensitivity and mild gastrointestinal upsets. At our present stage of knowledge, however, such assumptions have but little documented support.

It has been suggested that the cold agglutinins which appear so frequently during the late course of primary atypical pneumonia may promote hemolysis. Attempts have been made in this clinic and elsewhere to induce hemoglobinuria by chilling post-pneumonia patients with high titers of cold agglutinins but in no instances to my knowledge has hemolysis been observed. Cold agglutinins may also be demonstrated in certain cases of idiopathic hemolytic anemia. In some of these instances, chilling of the patient may have deleterious effects.

The rôle of the spleen in the destruction of red cells in health and disease has already been mentioned. Hemolytic anemias are occasionally observed, in which one or more lytic processes are apparently enhanced by splenic activity. The term "hyper-splenism" is being employed more and more to denote dyscrasias of this type. Frequently the spleen is enlarged and is the site of local disease such as tuberculosis, syphilis, giant-cell sarcoma, Hodgkin's disease or non-specific inflammatory changes (reticulo-endotheliosis) and it is assumed that in these disorders there is irritation and augmentation of splenic function. Symptomatic relief is afforded by splenectomy in some of these cases. Splenectomy should be considered a desperate therapeutic measure in acute hemolytic syndromes which persist despite transfusions and other supportive measures. Operation has proved successful even in cases with considerable hemolysins in the serum and may be justified by the assumption that splenic activity is an accessory factor in the total hemolytic picture.

STUDENT: What is Lederer's anemia?

DR. HANGER: It is a name applied to a febrile hemolytic syndrome characterized by severe hemoglobinemia with hemoglobinuria and a rapidly progressive anemia which develops suddenly in children and young adults. The condition is probably not a clinical entity. Autohemolysins may be demonstrated in the blood in some cases. It is important to remember that the hemolytic process may be terminated by the administration of normal blood. Patients with Lederer's syndrome may present an alarming picture but the outcome is often favorable if transfusions are promptly instituted and maintained.

SUMMARY

The normal mature red blood cell is a pliable biconcave disc composed of hemoglobin (25 per cent), stroma (3.5 per cent) and water (70 per cent). The arrangement of these elements in the cell is not well understood. Ordinarily, the life of an erythron is approximately 120 days and about 10,000,000 red cells are destroyed every second. Hemolysis may be said to exist when the normal rate of red blood cell destruction is increased. Although many agents may be responsible for hemolysis it is probable that, like saponins, all depend upon disruption of the cell membrane for their effect.

When hemolysis occurs rapidly, particularly if it be intravascular, free hemoglobin appears in the plasma above the normal concentration of 3 mg. per cent. Above levels of 135 mg. per cent, it appears in the urine. If tubular reabsorption is impaired hemoglobinuria may exist with considerably lower plasma levels. Usually, however, red cell destruction occurs in the reticuloendothelial system with the conversion of the hemoglobin to bilirubin and, when excessive, with the production of acholuric jaundice.

The breakdown of hemoglobin begins with oxidative ring rupture at the $\alpha$-methene group of the porphyrin fraction of the molecule, resulting in a green compound called verdohemoglobin. This loses its iron, which is stored in the form of hepatic and splenic ferritin, and becomes biliverdin which on reduction is called bilirubin. Bilirubin exists normally in concentrations of less than 1 mg. per cent in the serum and may be subdivided into two fractions: hemobilirubin and cholebilirubin. The former retains its globin, is non-dialyzable, does not pass the glomerular filter, produces the indirect van den Bergh reaction and represents the larger fraction. The latter is free of globin but is associated with plasma albumin and appears in urine when its concentration in the plasma exceeds 2 mg. per cent. In the intestinal tract bilirubin is further reduced to urobilinogen, part of which is absorbed into the portal circulation where it is almost entirely cleared by the liver and re-introduced into the bile. In the presence of impaired liver function, however, more than the usual 2 per cent of the portal bilirubin may elude the liver and gain access to the general circulation and so appear in the urine in increased amount.

Table I summarizes the usually recognized causes of red blood cell destruction while Table II attempts to classify these mechanisms as they occur in disease states.

How incomplete is our understanding, however, of the basic mechanisms of hemolysis is well illustrated in the case of sickle-cell anemia where a clear-cut abnormality of the red cell exists without obvious relation to the hemolytic process and where a rational form of therapy appears to have no effect. On the other hand, the excellent results obtained by splenectomy in congenital hemolytic icterus are seen to have some experimental foundation.

The clinical picture of chronic hemolysis is characterized by icterus, frequent enlargement of the spleen, anemia, leukocytosis and increase in reticulocytes. The urine is ordinarily free of bile although this is not invariable. The serum bilirubin is increased and classically gives an indirect van den Bergh reaction. Urobilin is increased in the stool and urobilinogen is usually present in the urine in abnormal amounts. If the liver is damaged, however, many of these points in differential diagnosis are of no value. Therapy is often disappointing unless some specific infection can be treated, some harmful agent be removed or splenectomy be advised. The success of the latter procedure is largely

limited to familial hemolytic jaundice although occasional excellent results are obtained in acquired hemolytic icterus and localized disease of the spleen.

Acute hemolysis presents quite a different and more serious prospect. The sudden onset is distinguished by headache, backache and leg pains. Pain in the abdomen may be severe and mimic an acute surgical abdomen. Chills and fever are common; shock, with anuria, may supervene. Anemia and hemoglobinemia are found. Hemoglobinuria may be present in severe cases or when tubular re-absorption is defective. Transfusion is the treatment of choice since it tends to overcome the anemia, correct the shock and so increase renal blood flow. In some instances it apparently arrests the hemolytic process as well. Alkalinization of the urine remains good therapy though perhaps of secondary importance in preventing anuria.

# Clinico-pathological Conference

## Diabetes, Hepatomegaly and Splenomegaly*

STENOGRAPHIC reports, edited by Robert J. Glaser, M.D., of weekly clinico-pathological conferences, held in the Barnes Hospital are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient was a sixty year-old white, married, investment banker who entered the Barnes Hospital for the first time on the Surigcal Service in July, 1924, because of recurrent attacks of abdominal pain. The family history revealed that one half-brother had diabetes. The findings of interest on this first admission were that the patient weighed 238 pounds and that his blood pressure was 158/100. Two urine specimens were negative for sugar. An appendectomy was performed and recovery was uneventful.

He reëntered the hospital on June 11, 1946, complaining of weight loss, frequency of urination and glycosuria. Ten years before his second admission a cataract was discovered in the left eye; it developed very slowly thereafter. Six years prior to entry he developed mild substernal pain which radiated down the left arm; he was seen by a cardiologist who told him that he had no heart disease. At that time roentgenograms of the gastrointestinal tract were negative.

The patient had learned to test his own urine for sugar years before admission and stated that for more than twenty years glycosuria had occurred intermittently without any other symptoms. Two months before the second admission he began to lose weight rapidly and he noted increased frequency of urination. One month later glycosuria became persistent and two weeks prior to entry he was seen by his physician who found the fasting blood sugar to be 263 mg. per cent. He was given a regulated diet and was advised to enter the hospital. The patient had taken moderately large amounts of alcohol for a number of years.

On admission, physical examination revealed the temperature to be 37°C., pulse 84, respirations 12 and blood pressure 110/68. The patient weighed 161 pounds. He was well developed and not acutely ill but showed evidence of recent weight loss. A partial cataract was noted in the left eye; both pupils reacted to light and accommodation. There was moderate arteriolar narrowing of the retinal arteries and some arterio-venous nicking. Examination of the upper respiratory tract was entirely negative. The lungs were clear to percussion and auscultation. The heart was not enlarged, the rhythm was regular and there were no murmurs; the second aortic sound was accentuated. The abdomen was protuberant. The liver edge was easily palpable 10 cm. below the right costal margin and was described as smooth and tender. The splenic tip was felt 2 cm. below the left costal margin. Vibration sense was diminished and there was hypesthesia to pin prick over the dorsum of each foot.

Laboratory studies were as follows: Blood count: red cells, 4,510,000; hemoglobin, 13.3 Gm.; white cells, 5,900; differential count: eosinophiles, 3 per cent; stab forms,

* From the Departments of Internal Medicine and Pathology, Washington University School of Medicine and the Barnes Hospital, St. Louis, Mo.

17 per cent; segmented forms, 45 per cent; lymphocytes, 32 per cent; monocytes, 3 per cent. Urinalysis: specific gravity, 1.026; albumin, negative; sugar, 3 plus; sediment, negative. Blood Kahn reaction: positive; quantitative Kahn test, 4 Kahn units; Kolmer-Wassermann test, negative. Stool: guaiac negative. Blood chemistry: nonprotein nitrogen, 19 mg. per cent; sugar, 145 mg. per cent; total protein, 5.5 Gm. per cent; albumin, 3.1 Gm. per cent; globulin, 2.4 Gm. per cent; cephalin-cholesterol flocculation test, 2 plus; bromsulfalein dye retention, 40 per cent in thirty minutes. Oral hippuric acid test: 50 per cent excretion as Na benzoate. Corrected sedimentation rate: 0.8 mm. per minute. Basal metabolic rate: plus 13. Electrocardiogram: T wave inverted in lead I; Q wave present in leads II, III and CF IV; S-T segment depressed in lead II; left axis deviation. Roentgenogram of the chest: "The cardiac silhouette is at the upper limit of normal. The left ventricle appears to be somewhat prominent. The hilar shadows are accentuated. The lung markings are coarse and extend far out into both fields." Gastrointestinal series: "Indeterminate." Oral cholecystograms: "Normal gallbladder."

On admission the patient was given a diet of 200 Gm. of carbohydrate, 150 Gm. of fat and 80 Gm. of protein. A mixture of 15 units of protamine zinc insulin and 30 units of regular insulin was given before breakfast. Choline chloride, skimmed milk, and intensive vitamin therapy were also prescribed. Because of persistent glycosuria the insulin dosage was increased so that the patient was taking a mixture of 85 units of regular and 35 units of protamine zinc insulin daily. On this schedule blood sugars were as follows: fasting, 79 mg. per cent; 11:00 A.M., 126 mg. per cent; 4:00 P.M., 167 mg. per cent. The urine sugar decreased to an occasional trace.

Four days after admission the patient developed sharp pain in the right upper quadrant and his temperature rose to 38.7°C. The pain was described as radiating through to the back and was relieved by codeine and aspirin. No changes were noted at this time in the physical findings. Further laboratory studies were as follows: white cells, 6,250; corrected sedimentation rate, 1.4 mm. per minute; Van den bergh test: direct, 0.72 mg. per cent; indirect, 0.8 mg. per cent; D-I ratio, 81 per cent.

The patient continued to have fever for the next ten days with frequent elevations to 39°C. His diabetes was difficult to control during this period and he had repeated insulin reactions. The temperature then fell to levels just above normal and the patient's condition improved. During the febrile episode he had had no jaundice or leukocytosis and the size of the liver and spleen had not changed. He was discharged on July 2, 1946, to return to the care of his private physician.

He did well for two weeks; he then developed profound anorexia, daily temperature elevations to 99° or 100°F. and increasing weakness. His appetite became so poor that he took nothing but orange juice by mouth and his daily insulin requirement was reduced considerably. Bilateral calf tenderness and extreme hypersensitiveness of the soles of the feet appeared, and the patient entered the hospital for the last time on August 2, 1946.

On admission, his temperature was 37.2°C., pulse 96, respirations 18, and blood pressure 142/88. He weighed 152 pounds. The significant changes from the physical findings previously recorded were as follows: a soft blowing apical systolic murmur was audible; the liver edge extended to the umbilicus and was quite tender; the spleen was questionably enlarged; the deep tendon reflexes were hypoactive and the soles of the feet were extremely painful to touch.

The laboratory findings were as follows: Blood count: red cells, 4,450,000; hemoglobin, 13.7 Gm.; white cells, 8,700; differential count: eosinophiles, 3 per cent; stab forms, 12 per cent; segmented forms, 53 per cent; lymphocytes, 26 per cent; monocytes, 6 per cent. Urinalysis: specific gravity, 1.015; sugar, trace; sediment, negative. Blood Kahn reaction: negative. Blood chemistry: non-protein nitrogen, 18 mg. per cent; icteric index, 3.7 units. Stool: guaiac negative. Prothrombin time: 93 per cent of normal. Electrocardiogram: "myocardial damage of the coronary type."

A Levine tube was passed and the patient was given eight feedings daily of a mixture consisting of 200 Gm. of protein, 300 Gm. of carbohydrate, and 75 Gm. of fat. Large amounts of vitamin B complex were added to the feedings and the patient was given an insulin mixture containing 20 units of protamine zinc and 60 units of regular. The fractional urines consistently showed large amounts of sugar and the insulin was increased until on the fifteenth day the patient was taking a mixture of 117 units of regular insulin and 48 units of protamine zinc insulin. On this dosage the urine became sugar free for the first time. During this period the patient had been essentially afebrile, his weight had remained stationary and the liver had not increased in size. Shortly after the peak of insulin dosage was reached, he began to have frequent reactions and the amount had to be decreased rapidly.

Laboratory studies at this time were: total protein, 4.4 Gm. per cent; albumin, 2.5 Gm. per cent; globulin, 1.9 Gm. per cent; calcium, 7.3 mg. per cent; phosphorus, 2.6 mg. per cent; alkaline phosphatase, 14 Bodansky units; carbon dioxide combining power, 60 volumes per cent; serum chloride, 81 milliequivalents/liter.

On the twentieth hospital day râles were heard at both lung bases. The heart was enlarged to percussion, the left border of dullness being at the anterior axillary line. One plus sacral edema and signs of ascites appeared. A repeat electrocardiogram showed little change. The patient was digitalized but signs of cardiac insufficiency persisted and he developed severe hiccoughs, often induced by palpation of the liver; they were unrelieved by sedation, atropine and carbon dioxide inhalation. Although his diabetes remained well controlled, he gradually became disoriented and then stuporous. During the last two weeks of life, the temperature gradually rose to a maximum of 40.8°c. and was uneffected by penicillin. The patient's course was steadily downhill. Cheyne-Stokes respirations were noted, the heart sounds became muffled and sacral and pretibial edema increased. Muscle twitching occurred but a Chvostek sign could not be elicited. Large doses of calcium lactate were given without effect. Sulfadiazine therapy was instituted and twelve hours later the temperature began to fall gradually. The patient's general condition showed definite improvement and on the day of death he seemed much better. That night, however, he suddenly became pulseless and despite emergency measures he expired quietly on September 1, 1946.

### CLINICAL DISCUSSION

DR. HARRY L. ALEXANDER: This case is extremely interesting and extremely complicated. For over twenty years the patient had had intermittent glycosuria, but until two months before the onset of his fatal illness, he had gotten on quite well. Suddenly the signs and symptoms of diabetes became prominent; that is, his glycosuria increased, frequency of urination appeared and marked weight loss occurred. It is well to inquire, therefore, whether such a chain of circumstances represents the effects of some important precipitating factor. Dr. Futcher, will you comment on this point?

DR. PALMER H. FUTCHER: Frequently when a patient with asymptomatic glycosuria or mild diabetes suddenly develops the symptoms and signs of severe diabetes, there is a precipitating cause and, under such circumstances, one thinks of concurrent disease, particularly infection. However, occasionally an increase in the severity of diabetes occurs without a demonstrable cause.

DR. ALEXANDER: In this case there was a noteworthy finding on the second admission, namely, hepatomegaly. The liver was described as being tender and smooth and impairment of liver function was indicated by significant bromsulfalein retention and a two plus cephalin-cholesterol flocculation test; on the other hand, the hippuric acid excretion was normal and so were the total and fractional proteins. Dr. Wade, would you suggest the nature of the liver involvement, if indeed there was disesae of the liver?

DR. LEO J. WADE: The only finding that suggests that this patient may have had a diffuse lesion of the liver is the bromsulfalein dye retention, but in view of the fact that the oral cholecystograms showed satisfactory dye concentration, I am skeptical of that result. I suspect that the most likely explanation for this single abnormality in liver function is a focal lesion of the liver, either infectious or neoplastic in origin.

DR. ALEXANDER: How do you interpret the two plus cephalin-cholesterol flocculation test? Is that equivocal?

DR. WADE: I think it is probably significant although a two plus cephalin-cholesterol flocculation test may occur in the absence of clinically obvious liver disease and under such circumstances no lesion may be found at autopsy to explain the abnormality. The alkaline phosphatase of 14 Bodansky units subsequently reported in this patient is compatible with a focal lesion of the liver and the absence of jaundice is not incompatible.

DR. ALEXANDER: Dr. Scheff, do you have any suggestions?

DR. HAROLD SCHEFF: I do not believe that cirrhosis of the liver can be ruled out.

DR. WADE: I would still be inclined to disregard the bromsulfalein retention in the absence of any other confirmatory test. The hippuric acid excretion does not suggest hepatic impairment.

DR. ALEXANDER: Forty per cent dye retention would seem to be a rather high figure.

DR. WADE: I have seen errors of this magnitude. Sometimes the amount of dye injected is excessive and occasionally the results are calculated incorrectly.

DR. ALEXANDER: Assuming the bromsulfalein retention is incorrect, and considering the two plus cephalin-cholesterol flocculation test and the elevated alkaline phosphatase, do you think that Dr. Scheff's suggestion of early cirrhosis may be correct?

DR. WADE: I do not believe so. At no time did the patient have anemia and normal total and fractional proteins would be unusual in a patient with cirrhosis. Cirrhosis is suggested by the dietary history but, of course, many individuals with a dietary history such as this man's do not develop cirrhosis.

DR. ALEXANDER: Could the large liver be explained on the basis of fatty infiltration such as is seen in association with diabetes?

DR. WADE: That is possible, but in such an instance I believe that the serum albumin would be decreased and that anemia would be present.

DR. SCHEFF: Splenomegaly is not seen in association with fatty infiltration of the liver.

DR. ALEXANDER: I agree that fatty infiltration *per se* would not explain the enlarged spleen.

DR. FUTCHER: In regard to the possibility that depressed liver function was as-

sociated with the fatty infiltration which occurs in diabetes, it is of interest to note the recent series of cases reported by Gray, Hook and Batty* who studied liver function in a number of patients with diabetes using the serum colloidal gold reaction. In those patients with severe diabetes the test was positive in 50 per cent of the cases. Dr. Wade has mentioned that a focal lesion may have been present here. I should like to ask specifically what type of lesion would lead to such marked hepatomegaly.

DR. WADE: During his hospital stay the patient had abdominal pain suggestive of that occurring with cholelithiasis.

DR. DONALD S. BOTTOM: The concentration of dye seen in the cholecystogerms is not very great but there is a very definite gallbladder shadow. In our experience we would say that there is a 70 per cent probability that the gallbladder was normal.

DR. ALEXANDER: Is it possible that this man had thrombophlebitis of the portal system at the time of his attacks of abdominal pain which led to his appendectomy in 1924 and of which subsequently there was recrudescence which gave rise to focal infection in the liver?

DR. WADE: I think the time interval is much too long.

DR. ALEXANDER: When the liver was palpated, the patient frequently developed hiccoughs. This suggests a lesion involving the diaphragm. Dr. Moore, do you have any suggestions?

DR. CARL V. MOORE: Considering only the clinical picture here it seems evident that the liver enlarged rapidly and that the tenderness was due to stretching of Glisson's capsule. Diaphragmatic irritation indicates enlargement upward as well as downward. I believe carcinoma of the liver, either primary or secondary, must be considered.

DR. ALEXANDER: Dr. Bottom, was the right diaphragm high?

DR. BOTTOM: It was slightly higher than the left diaphragm as is usually the case in normal subjects.

DR. W. BARRY WOOD, JR.: Most of the diagnostic suggestions considered so far do not explain the enlarged spleen. I should like to know how the spleen felt and just how large it was.

DR. WILLIAM H. OLMSTED: It was fairly firm and easily palpable below the costal margin.

DR. ALEXANDER: May not the spleen be enlarged in the presence of portal obstruction?

DR. WOOD: If the portal circulation is involved, the spleen as well as the liver is often affected. This point favors Dr. Scheff's suggestion of cirrhosis. If cirrhosis of the liver is to be considered, the fact that this patient also had diabetes suggests the possibility of hemochromatosis. However, there is no description of skin pigmentation.

DR. ALEXANDER: In hemochromatosis is not the liver smooth?

DR. OLMSTED: Yes, it is enlarged but usually quite smooth.

DR. WOOD: Is tenderness a prominent feature?

DR. OLMSTED: In the cases which I have seen the liver was not tender.

DR. WOOD: The presence of tenderness seems to me to mitigate strongly against hemochromatosis.

DR. ALEXANDER: What is the incidence of hemochromatosis without pigmentation of the skin?

DR. HENRY A. SCHROEDER: Approximately 20 per cent of patients with hemochromatosis do not have pigmentation of the skin.

DR. BERTRAND Y. GLASSBERG: The patient's diabetes was fairly well controlled

* GRAY, S. J., HOOK, W. and BATTY, J. L. Liver function studies in diabetes mellitus. *Ann. Int. Med.*, 24: 72, 1946.

during his hospital stay. In controlled diabetes hepatic enlargement is not common.

DR. ALEXANDER: That is especially true in patients given choline and other indicated therapy.

DR. SCHEFF: Is not fatty infiltration unusual in an older diabetic?

DR. OLMSTED: Yes.

DR. ALEXANDER: We now have suggestions of a focal lesion such as carcinoma, of cirrhosis and of hemochromatosis. It would be well now to mention the neurologic findings which were recorded. The patient had hypesthesia, paresthesias and later muscle tenderness and hyperesthesia over the feet. The reflexes were hypoactive.

DR. GLASSBERG: It has been pointed out that in carefully studied diabetic patients approximately 90 per cent exhibit some neurologic signs and these are often relieved by large doses of the vitamin B complex.

DR. OLMSTED: In my experience the neurologic symptoms are difficult to relieve.

DR. RAY D. WILLIAMS: I would agree. Large doses of B complex may sometimes help but they exert no specific effect. When the diabetes is controlled, improvement usually occurs. Cirrhosis has been mentioned as a possible diagnosis and it should be pointed out that although similar neurological findings are seen in cirrhotics, a high vitamin intake does not alleviate the complaints.

DR. ALEXANDER: The term "diabetic tabes" may be applied to a clinical picture such as the one exhibited here. However, there was also a positive Kahn test which brings up the possibility of syphilis. Subsequently the Kolmer-Wassermann test was negative and a quantitative Kahn test was reported as only four units. Dr. Clark, do you believe that a diagnosis of syphilis is justified?

DR. E. GURNEY CLARK: No, I think there is insufficient evidence for that diagnosis. A lumbar puncture was not performed, however, and valuable data would have been obtained from that procedure.

DR. ALEXANDER: Dr. Wade, do you believe that syphilitic involvement of the liver could give rise to the signs observed here?

DR. WADE: I think that is most unlikely.

DR. ALEXANDER: The description of the liver is not that of hepar lobatum. Further, in the late stages, the syphilitic liver is usually small.

DR. FUTCHER: Fever has been reported as an accompaniment of syphilis of the liver, but I do not know how well substantiated that observation is.

DR. VIRGIL C. SCOTT: About 12 to 15 per cent of patients with cirrhosis have low grade fever. In regard to syphilis of the liver, this patient received large amounts of penicillin which should have led to a satisfactory response had syphilis been the responsible factor.

DR. ALEXANDER: On the patient's second admission it is interesting to note that the total blood protein had fallen to 4.4 Gm. per cent, although the albumin-globulin ratio remained normal. The calcium likewise fell to 7.3 mg. per cent and muscular twitchings were noted. Dr. Futcher, do you believe that the fall in protein was responsible for the lowered calcium?

DR. FUTCHER: I think that that is entirely possible. If one attempts to explain the twitchings on the basis of hypocalcemia, however, it should be pointed out that no Chvostek sign was elicited.

DR. GLASSBERG: The low calcium would seem to be less important in view of the fact that the phosphorus was not elevated.

DR. ALEXANDER: Your point is well taken in that when the calcium falls, the phosphorus usually rises. Dr. Futcher, how do you explain the low serum chloride?

DR. FUTCHER: I am unable to do so particularly since there is no evidence of renal disease.

DR. ALEXANDER: Dr. Massie, on the basis

of the electrocardiograms and the signs of cardiac insufficiency, what changes would you expect in the heart?

Dr. Edward Massie: Certainly the patient had severe coronary artery disease as indicated by the electrocardiographic pattern. The terminal cardiac failure may likewise have been on this basis. It is further possible that the patient had a myocardial infarction shortly before death but this cannot be stated with any degree of certainty.

Dr. Alexander: Are there further suggestions?

Dr. Olmsted: Untreated diabetics are apt to have infection of various types.

Dr. Glassberg: Tuberculosis particularly should be considered here.

Dr. Alexander: The patient had unexplained fever which did not respond to penicillin; in addition, there was hepatosplenomegaly. Tuberculosis is certainly a worth while suggestion.

Dr. Wood: Tuberculosis is a definite possibility. Fever without leukocytosis is compatible with that diagnosis and it is well to point out that miliary tuberculosis may sometimes be extremely obscure.

Dr. Scheff: How would tuberculosis explain the severe abdominal pain?

Dr. Alexander: Perihepatitis perhaps might have been responsible.

Dr. Schroeder: The occurrence of heart failure in a patient with a poor dietary history also suggests the possibility of beriberi heart disease.

Dr. Alexander: In summary, this case presents a very difficult diagnostic problem. The patient had rather severe diabetes, probable diabetic neuritis, hepatosplenomegaly and fever. Among the explanations suggested for the hepatomegaly have been carcinoma of the liver, early cirrhosis and hemochromatosis. It is believed that the patient had cardiac failure probably due to coronary artery disease, possibly influenced by thiamine deficiency. Tuberculosis, perhaps miliary in distribution, may have explained the rapidly fatal course.

Clinical Diagnosis: Diabetes mellitus; diabetic neuritis; hepatomegaly and splenomegaly due possibly to carcinoma, cirrhosis, or hemochromatosis, and tuberculosis.

## PATHOLOGIC DISCUSSION

Dr. James O. Boley: At autopsy 50 cc. of fluid were present in the right pleural cavity and 25 cc. in the left pleural cavity. The heart was enlarged, weighing 610 Gm., and over the left ventricle there were depressed areas and thickening of the pericardium. On section these areas and the underlying muscle showed a large amount of fibrous tissue replacement. The myocardium varied in thickness from 5 to 16 mm. There was a healed infarct in the wall of the left ventricle measuring 6 by 8 cm. and extending into the interventricular septum to a distance of 3.5 cm. The coronary arteries showed advanced arteriosclerosis with marked narrowing of the left coronary. There was also advanced arteriosclerosis of the aorta. There were scars in the apices of both lungs and adhesions at the left apex. The lungs weighed 1,500 Gm., were firm and nodular and on cut section contained elevated reddish-gray areas. In the pleura near the right lower lobe, there was a calcified nodule 3 mm. in diameter, and there were also calcified nodules in the tracheobronchial and pulmonary lymph nodes.

The abdomen contained 1,200 cc. of fluid. The pancreas weighed 160 Gm. and was grossly normal. The kidneys together weighed 485 Gm.; the capsules stripped with little difficulty revealing yellowish-red, finely granular surfaces. An occasional small reddish-gray nodule was seen in the cortex.

The right adrenal gland weighed 48 Gm.,

the left 16 Gm. They were separated from the surrounding tissues with difficulty. Both were firm; on cut section the substance was rubbery, gray and translucent with occasional areas of caseation and foci of hemorrhage near the periphery. The changes were more extensive in the left adrenal than in the right.

The liver, which was yellowish-red in color, weighed, 2,930 Gm. On section the substance was mottled and occasional small gray nodules, up to 2 mm. in size, were noted.

The spleen weighed 660 Gm. A healed infarct was present. On section the substance was firmer than normal; the Malphighian bodies were increased in number and some were yellowish in color instead of gray. There was no generalized lymphadenopathy.

DR. MARGARET G. SMITH. We shall present the microscopic sections* in two parts, the first illustrating the vascular disease, and the second, the infectious process.

Figure 1 is a section of the descending branch of the left coronary artery, illustrating the marked arteriosclerosis and narrowing of the lumen. A section from one of the scarred areas (Fig. 2) shows a very old infarct with complete replacement by fibrous tissue. There were no changes suggesting recent infarction.

Sections from the pancreas revealed focal areas of interstitial fibrosis and hyalinization of most of the islands. In the kidney the changes of moderate arteriolar nephrosclerosis were seen.

Figure 3 is from the left adrenal and shows a large caseous area which extends to the fibrous capsule. There is infiltration by mononuclear cells, chiefly lymphocytes. In Figure 4, which is taken from the right adrenal gland, the process is seen to be less advanced. There are areas of caseous necrosis and, surrounding them, degeneration of cells of the adrenal and an accumulation of very large mononuclear cells. In Figure 5 granulomatous lesions with large mononuclear cells, suggestive of the epithelioid cells of tubercles, are seen. Surrounding these are zones of lymphocytes. A higher power view from this area (Fig. 6) shows a number of the large irregular mononuclear cells containing encapsulated organisms characteristic of Histoplasma capsulatum. In Figure 7 a low power view is seen; it does not show the characteristics of the organism but indicates the large numbers present. Another section (Fig. 8) taken from the capsule of the adrenal shows a proliferative type of lesion with miliary granulomas resembling tubercles.

The section pictured in Figure 9 is from the liver. Although in the gross only a few granulomatous lesions were seen, they are extremely numerous on microscopic examination and are seen in almost every field; some are circumscribed and some are seen in the portal spaces. They vary considerably in appearance, some having only a few large epithelioid cells loosely arranged with many lymphocytes, while in others the epithelioid cells are compactly arranged with a multinucleated giant cell in the center. Also to be noted are the infiltration of leukocytes in the sinusoids and the extremely large Kupffer cells.

In a section from the spleen the tubercles were grouped with numerous leukocytes about them. Many miliary lesions also were present in the lungs. On microscopic section they are seen in the septa and in the alveolar walls. (Fig. 10.) In most instances they are made up of irregular large mononuclear cells and a few lymphocytes, and on occasion multinucleated giant cells are present. In addition, there was an acute bronchopneumonia characterized by poly-

* Photomicrographs were made by the Departments of Illustration, Washington University School of Medicine.

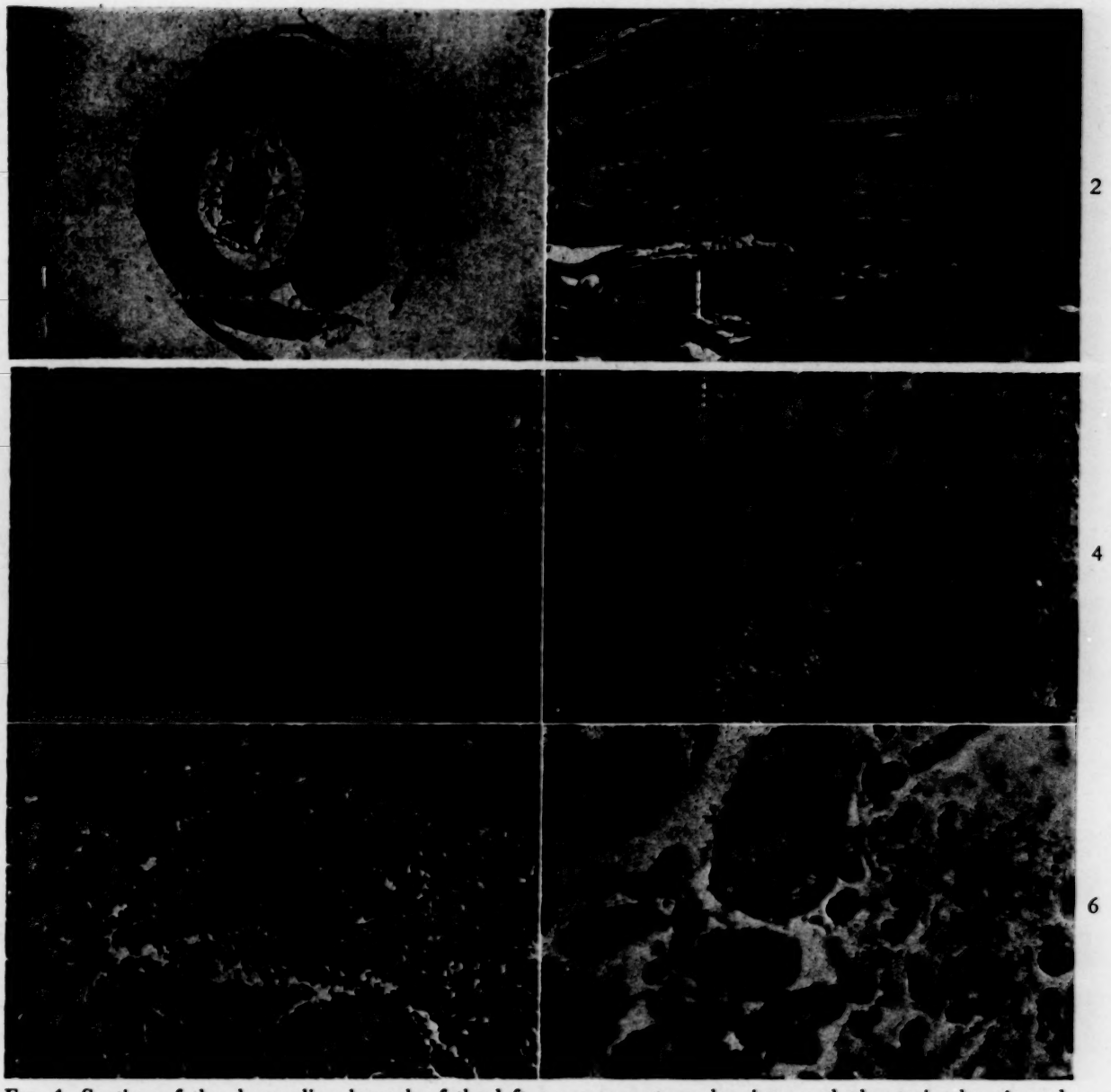


FIG. 1. Section of the descending branch of the left coronary artery showing marked arteriosclerosis and narrowing of the lumen.

FIG. 2. Section of the myocardium in an area of old infarction.

FIG. 3. Section from the left adrenal showing a large caseous area which extends to the capsule. There is cellular infiltration chiefly by lymphocytes.

FIG. 4. Section of the right adrenal gland showing changes similar to those in the left adrenal. The process is less advanced.

FIG. 5. Section from the adrenal showing granulomatous lesions.

FIG. 6. Higher power view of the area seen in Figure 5. The large mononuclear cells contain Histoplasma capsulatum.

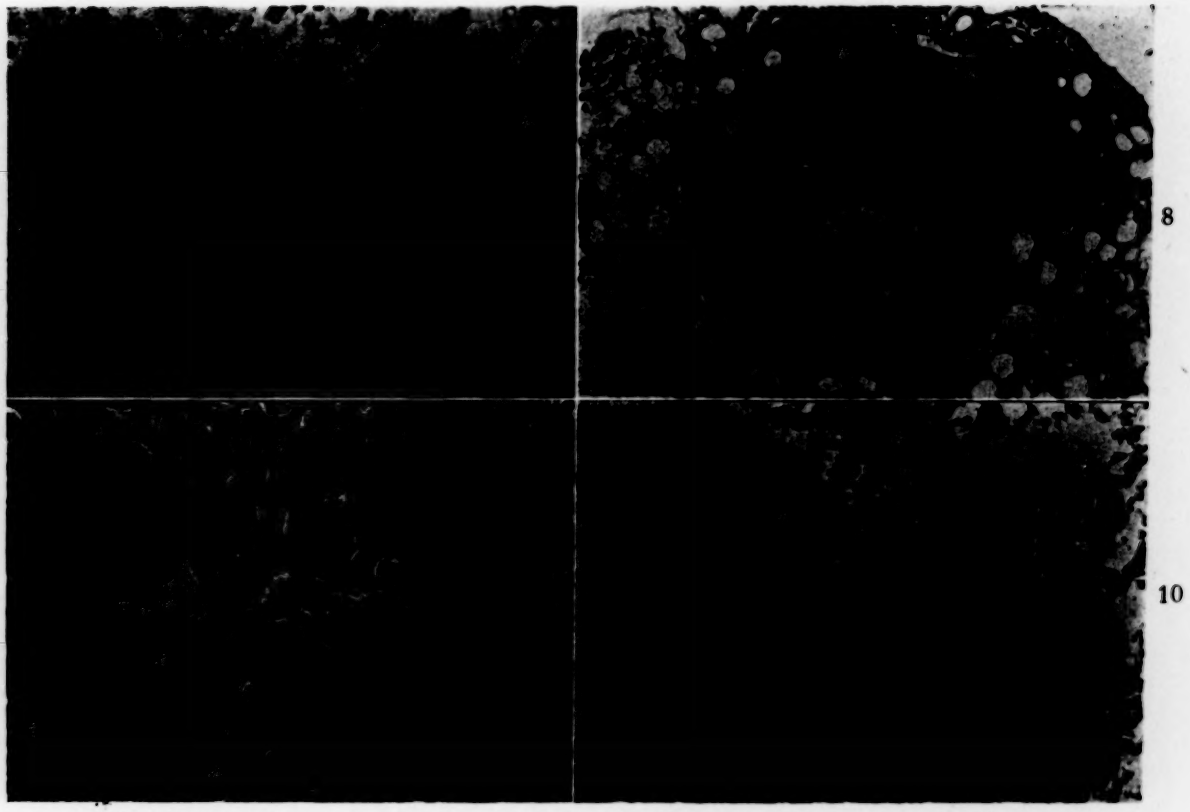


FIG. 7. View through the same area of Fig. 5 to indicate the large number of organisms present.
FIG. 8. Section from the adrenal showing a proliferative type of lesion with miliary granulomas resembling tubercles.
FIG. 9. Section from the liver showing numerous granulomatous lesions. Note the infiltration of leukocytes in the sinusoids and the extremely large Kupffer cells.
FIG. 10. Section from the lung showing granulomatous lesions in the septa and in the alveolar wall.

morphonuclear infiltration and fibrin. A few granulomatous lesions were present in the interstitial tissue of the kidney. (Fig. 11.)

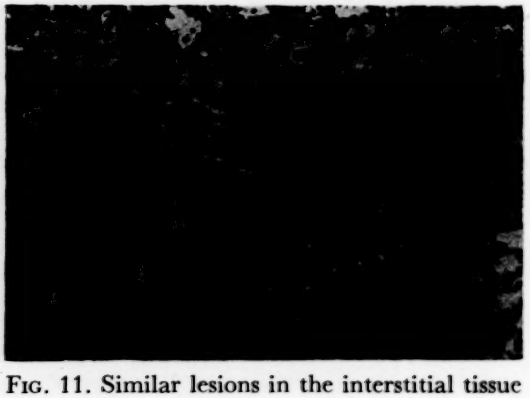

FIG. 11. Similar lesions in the interstitial tissue of the kidney.

There was no generalized lymph node enlargement; however, several bronchopulmonary nodes were sectioned and a few granulomatous lesions were found near the capsule. A great deal of anthracosis was present and the reaction was believed to be due principally to anthracosilicosis.

In the study of sections from organs other than the adrenals, Histoplasma capsulatum could not be identified with certainty although in a number of the lesions a single body, resembling the organism, was seen. It is believed, however, that all the granulomatous lesions were caused by the same disease process.

As far as the duration of the illness is concerned, the lesion in the left adrenal was much older than that in the right. The lesions in the other organs are of approximately the same age as those in the right

adrenal. The oldest are compatible with a year's duration. An infarct in the adrenal gland probably occurred within five or six months of death and may explain the onset of the clinical manifestations of the disease.

DR. ALEXANDER: Dr. Bottom, is there much calcification visible in the chest film?

DR. BOTTOM: It is seen only in one small area.

DR. ALEXANDER: Is not the incidence of pulmonary calcification high in association with positive skin tests with histoplasmin?

DR. SMITH: Yes.

DR. VIRGIL C. SCOTT: Dr. Smith, how many cases of histoplasmosis have you seen at autopsy?

DR. SMITH: Our first case was seen in 1938 in an adult with histoplasmotic endocarditis.* We have also seen two cases in children. Since 1936, however, there have been about twelve cases reported in this section of Missouri and the adjacent portions of Illinois.

*Pathologic Diagnosis:* Fibrocaseous histoplasmosis of the adrenals: miliary granuloma of the liver, spleen, kidney, lung, bronchopulmonary lymph node, bone marrow and pancreas; bronchopneumonia of the upper and lower lobes of the right and the lower lobe of the left lung; arteriosclerosis of the coronary arteries, advanced, with narrowing of the anterior descending and left circumflex branches; healed infarcts of the posterior and anterior wall of the left ventricle, and the anterior part of the interventricular septum; arteriolar nephrosclerosis, moderate; ascites (1,200 cc.); hyalinization of the islands of Langerhans (history of uncontrolled diabetes); arteriosclerosis of the aorta, advanced with ulceration; of the splenic and superior mesenteric arteries, moderate; of the renal, hepatic, inferior mesenteric and pulmonary arteries, slight; anthracosilicotic nodules in the middle and lower lobes of the right lung and bronchopulmonary node.

* BEAMER, P. R., REINHARD, E. H. and GOODOF, I. I. Vegetative endocarditis caused by higher bacteria and fungi. *Am. Heart J.*, 29: 99, 1945.

# Case Report

# The Electrocardiographic Diagnosis of Acute Myocardial Infarction in the Presence of the Wolff-Parkinson-White Syndrome*

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NEW YORK, NEW YORK

THE syndrome of the short PR interval with prolonged QRS complex is apparently a benign congenital anomaly which was first described in 1930 by Wolff, Parkinson, and White.[1] The essential criteria which have been established[2] as diagnostic of this electrocardiographic abnormality, all of which must be present, include a PR interval of 0.10 second or less, a QRS complex of 0.10 second or more, and slurring of the initial ventricular deflection. In addition, reversal of the above picture to normal is commonly observed either spontaneously, after exercise, or after the administration of certain drugs (atropine, quinidine[3,4,5]); and paroxysmal tachycardias, either supraventricular or ventricular, frequently occur. Furthermore, this syndrome has been most frequently observed in healthy young males without evidence of organic heart disease.

From its recognition in 1930 to about 1940, investigations of this syndrome were focussed on determining its mechanism, and appear to have established satisfactorily its origin in a structural anomaly which provides an alternative route for conduction of impulses from the sinus node to the ventricles.[6,7,8] Since 1940, increasing emphasis has been placed on the coexistence of this physiologic peculiarity and various forms of organic heart disease. At present there are few reports on its association with acute myocardial infarction, a situation of special interest because the diagnosis of the latter is rendered difficult by the basic electrocardiographic changes of this syndrome. We have found reports of only two cases in which the possible coexistence of acute myocardial infarction and the Wolff-Parkinson-White syndrome is considered.[9,10] We are, therefore, presenting an additional case in which the likelihood of this association was important, and which illustrates the difficulties in the differential diagnosis of acute myocardial infarction by means of the electrocardiogram under these circumstances. The usefulness of the electrocardiogram in the diagnosis of acute myocardial infarcation is almost wholly vitiated by the presence of the Wolff-Parkinson-White syndrome.

## CASE REPORT

The data submitted below are drawn from records of three hospital admissions, visits to the cardiac clinic, and reports from Dr. Sidney H. Schechner.

The patient, N. S., a fifty-four-year old white male, was first admitted to Beth Israel Hospital on January 2, 1943, because of substernal oppression of four hours' duration. This symptom

* From the Cardiac Service, Beth Israel Hospital, New York, and the Department of Pharmacology, Cornell University Medical College, New York.

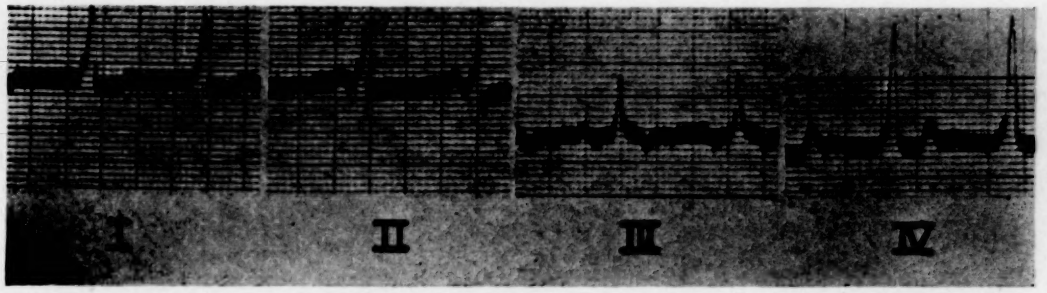


FIG. 1. Tracing taken on April 24, 1941, showing a short P-R interval and prolonged QRS complex with slurring of $R_1$ and $R_2$ (four standard leads).

was associated with profuse sweating, dyspnea, several episodes of vomiting, and an ashen grey appearance of the face. Two doses of morphine sulfate, 0.015 Gm., subcutaneously ten minutes apart had been given by his physician. Five years previously a similar episode of tachycardia and chest pain had been relieved by a subcutaneous injection of morphine sulfate, 0.015 Gm. Ever since the first attack, the patient had complained of substernal pain on walking a few blocks, which was promptly relieved by rest. The earliest available electrocardiogram (Fig. 1) taken on April 24, 1941, was characteristic of the Wolff-Parkinson-White syndrome.

On admission the patient appeared acutely ill. The temperature was 99°F. and the respiratory rate was 26 per minute, with moderate dyspnea. The skin was cool and the facies ashen grey. The lungs were clear. The heart was not enlarged. The heart sounds were of poor quality. The rhythm was irregular. The ventricular and pulse rates were 144 per minute. There was no enlargement of the liver or spleen, nor any peripheral edema.

The pertinent findings in the subsequent course are shown in Fig. 2. It may be seen that the temperature rose to 100.2°F. on the first day of illness, then fell to normal where it remained for the rest of the hospital stay. The rhythm became regular and the pulse rate dropped to 84 per minute on the first hospital day. The blood pressure, which was 114/70 on admission, dropped gradually to 80/50 on the fourth hospital day and then recovered so that at the time of discharge it was 115/70. The white blood cell count on admission was 14,000 per cu. mm. with 84 per cent polymorphonuclear leucocytes; the blood sedimentation rate was 1 mm. at the end of one hour. Subsequent blood counts on the sixth and eleventh hospital days revealed 8,900 leucocytes per cu. mm. with 71 per cent neutrophiles, and 7,000 leucocytes per cu. mm. with 77 per cent neutrophiles, respectively. A blood sedimentation rate on the ninth hospital day was 3 mm. at the end of one hour. Electrocardiograms taken on the second, eleventh, and thirty-first hospital days showed a short PR interval with prolonged QRS complex with serial changes in the T waves, namely, inversion of $T_2$ and $T_3$. (Fig. 3.) The second tracing, which is not illustrated, showed changes similar to those seen in the third electrocardiogram. (Fig. 3B.)

The hospital course was uneventful. Because of the rise in temperature, leucocytosis, fall in blood pressure and serial changes in the electrocardiogram, a diagnosis of arteriosclerotic heart disease with acute myocardial infarction was made. The patient was discharged to his home on February 14, 1943.

The patient was well until June 8, 1944, when following excitement he experienced a persistent stabbing pain under the left nipple. He was given a hypodermic injection of morphine sulfate, 0.015 Gm., with ensuing marked relief of pain. He was admitted to Beth Israel Hospital two hours after the onset.

At this time, the patient appeared moderately ill. The temperature was 99°F. There was cyanosis of the lips and orthopnea. The neck veins were slightly distended in the upright position. There were occasional moist râles at the base of the right lung. The heart was not enlarged. The heart sounds were of poor quality and tic-tac in nature. The blood pressure was 114/82. The ventricular rate was 230 per minute and the

pulse rate 180 per minute. The rhythm was irregular. The electrocardiogram at this time showed rapid auricular fibrillation with ventricular premature contractions. (Fig. 4A.) The white blood count was 11,300 with 77 per cent neutrophiles on the second day of the illness. A thirteenth day of illness was 5 mm. at the end of an hour.

The patient received quinidine sulfate, 0.6 Gm., daily for the first ten hospital days. The patient was discharged to his home on June 30, 1944.

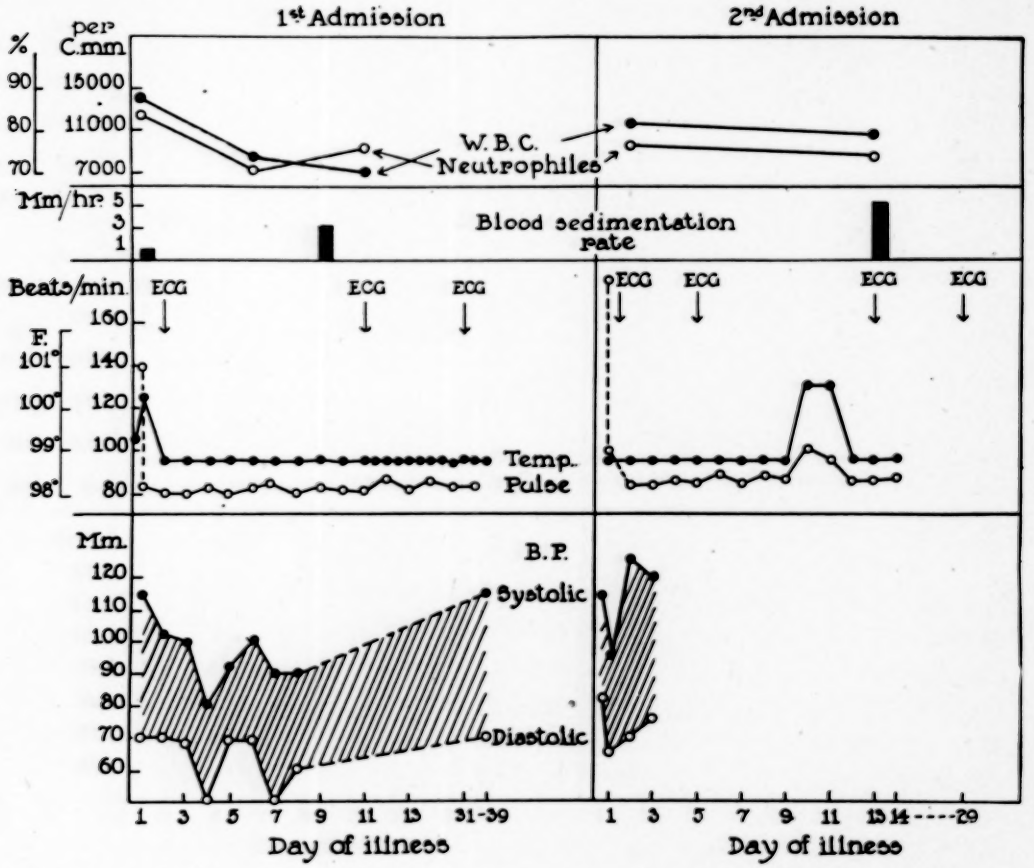


FIG. 2. Chart showing clinical data obtained during the first and second hospital admissions.

second blood count on the thirteenth hospital day showed 10,600 with 73 per cent neutrophiles.

The patient was afebrile throughout the hospital course except for an unexplained rise of temperature to 100.4°F. on the tenth and eleventh hospital days. The blood pressure fell to 96/66 with a regular rhythm and ventricular and pulse rates of 100 per minute several hours after admission. On the second day, the blood pressure rose to 126/70 with pulse and ventricular rates of 84 per minute. Subsequent electrocardiograms (Fig. 4B) revealed the syndrome of short PR interval with transient changes in the T waves similar to those seen during the first admission. The blood sedimentation rate on the

The third admission on July 8, 1946, was primarily for the purpose of trying to convert the abnormal rhythm to a normal sinus rhythm by means of atropine or quinidine. On July 9, 1946, a dose of 0.002 Gm. of atropine sulfate was given subcutaneously. Five minutes after its administration, a middle nodal tachycardia with normal QRS complex was present. (Fig. 5B.) Ten minutes after administration of the atropine, the short PR interval with wide QRS complex had reappeared. (Fig. 5C.)

During this admission, the patient was given and has been receiving to date a daily dose of 3 Gm. of quinidine sulfate orally. This amount proved insufficient to cause a reversion of the

FIG. 3. Tracings taken during the first admission showing serial changes in the T waves. A, third day of illness. All the T waves are upright; B, thirty-first day of illness. $T_2$ and $T_3$ are now inverted.

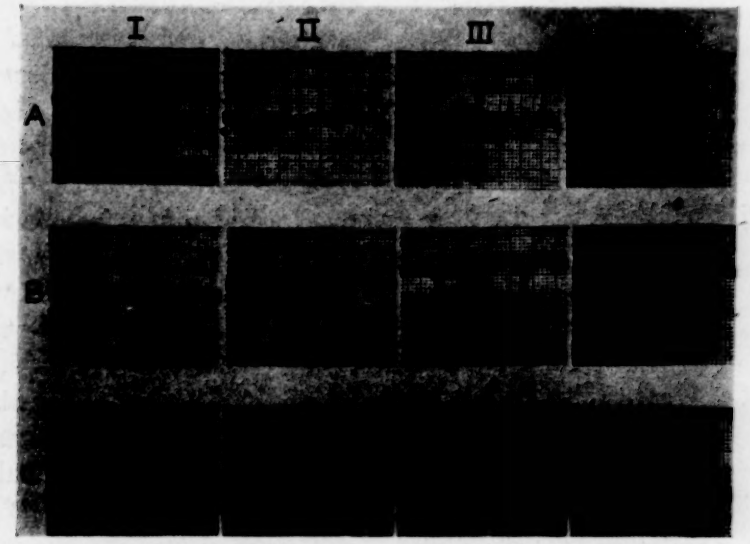


FIG. 4. Tracings taken during the second hospital admission showing a rapid paroxysmal rhythm followed by transient variations in the contour of the T waves. A, first day of illness. Auricular fibrillation (ventricular rate 150 per minute) with ventricular extrasystoles. Note the normal QRS complex time; B, fifth day of illness. $T_1$ and $T_2$ are diphasic. $T_3$ is upright, $T_4$ is inverted; C, thirteenth day of illness. $T_1$ is still diphasic, $T_2$ and $T_3$ are now inverted and $T_4$ is upright.

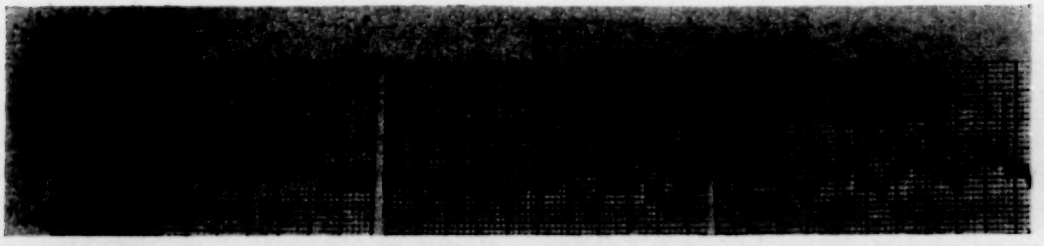

FIG. 5. Influence on the rhythm of atropine sulfate, 0.002 Gm., subcutaneously (lead II only). A, control; B, five minutes after atropine showing a nodal rhythm; C, ten minutes after atropine showing return to the Wolff-Parkinson-White syndrome.

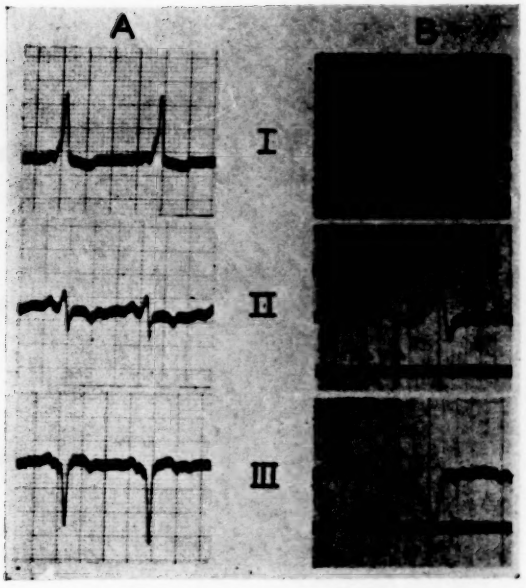


FIG. 6. Comparison of slow (25 mm. per second) and fast (75 mm. per second) moving film (three standard leads) to demonstrate easier recognition of the Wolff-Parkinson-White syndrome. The arrows indicate the P-R interval which measures 0.08 to 0.09 seconds in all leads. A, slow film; B, fast film.

rhythm to normal, although it has prevented a recurrence of any rapid paroxysmal rhythm. All of the tracings since his discharge on July 20, 1946, have shown the Wolff-Parkinson-White syndrome.

## COMMENT

In this case it is interesting that the diagnosis both of the Wolff-Parkinson-White syndrome and of acute myocardial infarction was questioned.

*Diagnosis of Wolff-Parkinson-White Syndrome.* We encountered the argument that the electrocardiogram in this case did not fulfill the essential diagnostic criteria which we have outlined above, because the PR interval in Lead III (Figs. 1 and 6A) appeared to be as long as 0.16 seconds. However, when tracings were taken on a rapidly moving film with camera speed of 75 mm. per second (Fig. 6B) the clearer detail revealed that the isoelectric PQ phase in Lead III actually belonged to the QRS complex rather than to the PR interval. Thus in this lead also, shortening of the PR interval and widening of the QRS time was demonstrable. It is important to emphasize that an apparently normal PR interval with a QRS complex of normal duration in one or two leads does not preclude a diagnosis of the Wolff-Parkinson-White syndrome, provided at least one standard lead conforms to the criteria which we have listed. Thus it would seem that to allocate correctly an initial isoelectric phase of the QRS complex, the PR interval should be measured in that lead in which it is shortest, and the QRS time in that lead in which it is longest.

*Diagnosis of Myocardial Infarction.* The serial changes in the electrocardiograms of the first hospital admission (Fig. 3) were accepted at that time as proof of a recent myocardial infarct. However, the fact was overlooked that this criterion, which is usually most reliable in the diagnosis of acute myocardial infarction, namely, serial changes in the T waves, fails to apply in the presence of the Wolff-Parkinson-White syndrome because of the spontaneous inversion of the T waves which occurs frequently in these cases in the absence of any other evidence of a myocardial infarct.[11]

Furthermore, recent studies [12,13,14] indicate that the cessation of a rapid paroxysmal rhythm in patients without organic heart disease may be followed by an electrocardiographic picture that resembles that of myocardial infarction with persistence of the abnormal T waves for as long as several weeks.[12] It should be noted that in the case we are reporting, the patient was admitted twice to the hospital following a rapid paroxysmal rhythm. (Fig. 4A.)

It is obvious that both the spontaneous changes in the T waves in the Wolff-Parkinson-White syndrome and the occasional persistence of an abnormal electrocardiogram after the cessation of a rapid paroxysmal rhythm vitiate the usefulness

of serial changes in the T waves in the diagnosis of acute myocardial infarction. This conclusion is in harmony with that of Goldbloom and Dumanis[10] who, on the basis of the general clinical picture, made a diagnosis of acute myocardial infarction in a thirty-three year old man with the Wolff-Parkinson-White syndrome. They noted that the serial changes in the electrocardiogram might not be significant because of the well-known "spontaneous variability of contour of the T wave" in this syndrome. Eichert[9] has also called attention to the fact that the electrocardiographic peculiarities of the Wolff-Parkinson-White syndrome may simulate those of acute myocardial infarction.

In view of the above spontaneous variations in the T wave in the Wolff-Parkinson-White syndrome, we hoped that in the case of our patient some aid in the diagnosis might be secured from the electrocardiogram if a normal sinus rhythm could be restored by atropine or quinidine. Unfortunately, the restoration of a normal rhythm was not accomplished. However, it is interesting that a middle nodal tachycardia was induced by atropine (Fig. 5B), an effect which is compatible with Katz's hypothesis[15] of the origin of the Wolff-Parkinson-White syndrome in a coronary nodal rhythm rather than in an aberrant pathway from the sinus node.

Confirmatory evidence for the diagnosis of a myocardial infarction which results in either right or left bundle branch block may be afforded by a lengthening of the interval from the initial to the intrinsic deflection of the QRS complex on the involved side, as shown in the six standard precordial leads.[16] In the case which we have reported, however, this technic failed to reveal any difference in the above intervals when the precordial leads from the right and left sides of the heart were compared. In Fig. 7 it may be seen that this interval in leads CF I to CF III (right side) measured 0.07 to 0.08 seconds, and in leads CF IV to CF VI (left side), 0.08 to 0.09 seconds. These findings probably rule out the diagnosis of a bundle branch block on an organic basis. Boyer[16] also studied these measurements in a case of the Wolff-Parkinson-White syndrome and found that the above interval was equal and normal (0.08 seconds) in the precordial leads from the right and left sides of the heart.

FIG. 7. Precordial leads $CF_1$ to $CF_6$. The arrows indicate the interval between the beginning of the QRS complex and the start of the intrinsic deflection (the first rapid downward movement of the string). Absence of organic lesions in the bundles is indicated by the fact that this interval is approximately the same (0.08 ± seconds) in the leads taken from the right (CFI to III) and left (CGIV to VI) sides of the heart.

In finally evaluating the cardiac diagnosis in this case, we are forced, as we have shown, to discard the electrocardiogram and to rely on the clinical picture and on other laboratory tests. In the first hospital admission, the clinical course presented the classical picture of an acute myocardial infarct, with sudden onset of prolonged substernal oppression with vomiting, collapse symptoms, and a gradual fall of blood pressure to 80/50 on the fourth day of illness. At the onset, fever and leucocytosis were also present. The only laboratory examination which failed to confirm the diagnosis of acute myocardial infarction was the blood sedimentation rate, and it is

well known that an elevation of this value is not essential to the above diagnosis. Therefore, it is quite likely that a fresh myocardial infarction took place just before the first hospital admission. In the second hospital admission, the picture is not so clear-cut, but the symptomatology can be adequately explained by an attack of paroxysmal auricular fibrillation with some degree of left ventricular failure.

## SUMMARY AND CONCLUSIONS

1. A case is presented which illustrates the special problems involved in the electrocardiographic diagnosis of acute myocardial infarction in the presence of the syndrome of short PR interval and prolonged QRS complex.

2. The electrocardiographic changes of acute myocardial infarction may be so masked or obscured by the spontaneous variations in the T waves in the Wolff-Parkinson-White syndrome that the electrocardiogram loses its value in the diagnosis of the former condition.

3. Hence, the diagnosis of acute myocardial infarction in the presence of the Wolff-Parkinson-White syndrome can be established only on the basis of criteria other than the electrocardiographic changes.

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# American Federation for Clinical Research

## ABSTRACTS OF PAPERS PRESENTED AT THE ANNUAL MEETING HELD IN CHICAGO, APRIL 28, 1947

## Electrophoretic Study of Sera from Patients with Pinta and Yaws

M. L. Dillon, M.D.

*Durham, N. C.*

(Introduced by Grant Taylor, Ph.D.)

It has been demonstrated by Cooper, Rein and Beard with sera from two patients with kala-azar that the presence of hyperproteinemia, hyperglobulinemia, or hypergammaglobulinemia could not be used to prove or disprove the specificity of positive serological reactions for syphilis. This report extends the above work by a study of sera from three patients with pinta and three patients with yaws. Electrophoretic studies and serodiagnostic tests were performed, protein concentrations were determined and again it was demonstrated that there is no correlation of the serodiagnostic tests with hyperproteinemia, hyperglobulinemia or hypergammaglobulinemia.

## Effect of Penicillin on the Transient Bacteremia Following Dental Extraction

Robert J. Glaser, M.D., *and* (*by invitation*) Arnold Dankner, M.D., Sydney B. Mathes, M.D. *and* Carl G. Harford, M.D.

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This study was undertaken to determine the effect of penicillin on the transient bacteremia known to follow dental extraction. It was considered that such a study would aid in the evaluation of the prophylactic use of penicillin in patients with rheumatic and congenital heart disease who require dental operations.

Blood cultures were taken before and immediately after extraction of one or more teeth in two series of patients, each forty in number. In the first, or control series, alpha-hemolytic streptococci or non-hemolytic streptococci were recovered from over 60 per cent of the postextraction cultures. In the second series, the patients were given large doses of penicillin over a twenty-four-hour period prior to extraction. In this group, approximately 40 per cent of the postextraction cultures were positive for alpha-hemolytic streptococci or non-hemolytic streptococci. In the control series the former organism predominated, whereas in the penicillin series the latter organism predominated.

The use of penicillin did not result in a significant decrease in the occurrence of bacteremia after extraction of teeth in patients with normal gums; however, in those with gingivitis or pyorrhea a definite decrease in incidence of positive blood cultures was noted. Indeed, the over-all reduction of positive blood cultures with the use of penicillin was due entirely to a decrease in the incidence of bacteremia following extraction of teeth from patients with gingivitis or pyorrhea.

It was concluded from this investigation that prior to extraction of teeth in patients with rheumatic or congenital heart disease, penicillin should be given for at least twenty-four hours in high dosage to those patients with gingivitis or pyorrhea and should be continued for two or three days after extraction. In patients whose gums are normal penicillin may be begun immediately prior to extraction but should be continued for approximately two to three days. It is postulated that the use of penicillin for two or three days following extraction represents, in effect, the earliest possible treatment of bacterial endocarditis if it becomes established following extraction. Whether this method will prove totally effective awaits further experience.

## Rheumatic-like Lesions Found in Unselected Autopsies

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A clinical pathological study was made of hearts from 145 unselected autopsies under conditions favorable to unbiased observations. The results of this study may be summarized as follows:

1. Rheumatic or rheumatic-like microscopic nodules were found in almost 52 per cent of the hearts. These nodules occurred in various parts of the heart, most frequently in the myocardium, atrial endocardium, and annuli and spongiosa

of the mitral and aortic valves. There was no sharp line of differentiation between rheumatic and rheumatic-like nodules.

2. Fifty per cent of the hearts with typical rheumatic nodules were obtained from patients fifty years or more of age and a number of typical rheumatic nodules were observed in those hearts from patients seventy years of age or older.

3. Rheumatic or rheumatic-like nodules were seen most commonly in association with bacterial infections, the presence of bacteremia (not necessarily beta hemolytic streptococcic), clinical rheumatic fever or rheumatic histories.

4. Gross rheumatic or rheumatic-like valvular lesions were observed in 48 per cent of the 145 hearts. The hearts with these gross lesions had more rheumatic or rheumatic-like microscopic changes than the hearts without gross valvular lesions.

5. Based on these observations, it seems logical to assume that a rheumatic-like carditis is more frequent than is generally believed. It is not necessarily restricted to younger age groups; it may be slight, moderate or marked and may lead to deformity or heal with minimal or no deformity. This carditis was found associated most frequently with bacterial infections, especially bacteremias, not necessarily beta-hemolytic streptococcic.

## Fibrinolysin Production by β-Hemolytic Streptococci

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Although little is known concerning the rôle of streptococcal fibrinolysis in the pathogenesis of disease, there is some indication that β-hemolytic streptococci which produce large amounts of fibrinolysin are usually associated with severe infections, whereas little fibrinolysin is produced by strains from mild infections. In order to investigate this relationship further a technic for measuring the amount of fibrinolysin produced by β-hemolytic streptococci was devised. Many strains of streptococci isolated from the nasopharynx of patients with respiratory disease and from normal subjects were studied by this method. Strains of group A streptococci isolated in various parts of the United States were similarly examined.

Analysis of the results of these tests showed that the ability to produce fibrinolysin varied according to the Lancefield group and that in general those groups commonly considered to be pathogenic produce considerable amounts. Group A streptococci appeared to vary in their fibrinolytic capacity according to the Lancefield type of organism. Organisms of one type seemed to exhibit a similar fibrinolytic capacity irrespective of the section of the country from which they were derived. No relationship was apparent between the severity of the disease and the fibrinolytic ability. A correlation was demonstrated between the amount of fibrinolysin produced and the ability of the strain to stimulate antifibrinolysin in the patient.

## Endemic Influenza

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Until recently, it had been widely believed that influenza was essentially, if not entirely, an epidemic disease. However, many reports of the occurrence of sporadic cases of influenza A and influenza B have suggested that influenza viruses A and B are in constant circulation in a population and that cases of influenza would be found repeatedly in non-epidemic periods if a search were made. The result of such a continuous search for cases of influenza over a period of nearly three and one-half years at Fort Bragg, N. C., is the subject of the present report.

Respiratory disease admissions to the Station Hospital were the source of cases. Acute and convalescent phase sera were obtained from each patient and tested for antibodies to influenza viruses A and B. The diagnosis of influenza was made when a fourfold or greater increase in titer of antibodies occurred. The study was started November, 1942 and terminated March, 1946. A total of 2,932 respiratory admissions was studied and those patients selected included all types of respiratory disease.

Influenza A and influenza B occurred sporadically as well as in epidemic form. One localized outbreak of influenza B occurred. It was found that cases of influenza occurring in non-epidemic periods were very difficult or impossible to recognize clinically.

The conclusion was reached that influenza may be viewed as an endemic disease which periodically erupts in epidemic form.

## Streptomycin Treatment of Tularemia

John B. Johnson, M.D., *and* (*by invitation*) Charles B. Wilkinson, M.D. *and* Edmundo Figueras, M.D.

From the Department of Medicine, Howard University School of Medicine, Washington, D. C.

This report summarizes the results obtained in five patients with tularemia who were treated with streptomycin. Three of the cases were of the ulceroglandular type and two were of the pulmonic type. The latter and one of the former were critically ill on admission. In two cases the streptomycin was started during the first week of the disease, in two others it was started in the third week and in one, the ninth week of the disease. The daily dose of streptomycin was 0.4 Gm. in two cases and 0.8 Gm. in three cases. The drug was continued until the temperature was normal for several days. In each instance there was a sharp drop in temperature within twenty-four hours after the institution of treatment. The temperature reached and remained normal on the fourth day in one patient, on the twelfth day in three patients and on the eighteenth day in one patient. All patients showed rapid subjective improvement.

Two of the three patients with the ulceroglandular type of tularemia required surgical drainage even though they were given streptomycin into the bubo as well as by intramuscular injection. In these two patients treatment was not started until the third and ninth week of the disease. In the patient whose lymphadenopathy resolved without drainage treatment was started in the first week of the disease.

One patient who had been pregnant for three months and whose disease had gone untreated for nine weeks aborted after three weeks of streptomycin therapy. Pathological examination of the placenta and fetus showed no changes which could be attributed to streptomycin or tularemia.

One patient, a chronic alcoholic, developed delirium tremens. Treatment had to be resumed in one patient with pneumonic involvement because of a recurrence of fever. This was true even though this patient had received 12.8 Gm. of streptomycin in sixteen days. One patient developed an increase in fever on two occasions while streptomycin was being given; in each instance the temperature fell following penicillin therapy.

Streptomycin was considered very effective therapy in these five cases as determined by the reduction in days of fever, bed ridden days and duration of buboes. In the two cases where streptomycin was started during the first week of the disease, the agglutination titer was 1:40 or less on admission. In each instance a rising titer of agglutinins occurred suggesting that the streptomycin did not interfere with antibody formation.

## Clinical Problem of Pheochromocytoma

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A study was made of sympathetic tumors of the adrenal gland and four cases were reported which showed the various clinical manifestations of this disease. Since vasomotor symptoms were prevalent in all these cases, one must consider an adrenal tumor in patients presenting these complaints irrespective of the severity of the symptoms. If the correct diagnosis is made, as in Case I, (and this is now possible with newer diagnostic procedures such as histamine and mecholyl) and if the patient's cardiac reserve is sufficient to tolerate the operation and its hypertensive reaction, a clinical cure results. If the correct diagnosis is not made fixed hypertension with progressive cardiac and renal failure results (Case II). If an adrenal tumor is found, as in Case III, the true nature of the tumor should be determined before operation since the anaesthesiologist and surgeon will then be aware of

the problem and better able to plan the anesthesia, avoiding spinal, and at operation proceed with dispatch to remove the tumor. Should an unusual hypertensive reaction be observed by the anesthesiologist during the course of an elective operation, as in Case IV, the diagnosis of sympathetic adrenal tumor should be considered and if proven by abdominal exploration serious consideration should be given to its removal.

The use of epinephrine, in the event of a shock state with pulmonary edema, during the operation for removal of these adrenal tumors is not judicious since actually the state of shock is best explained as the result of left ventricular heart strain secondary to increased peripheral resistance owing to an excess circulatory epinephrine. Therapy of the condition includes rapid removal of the tumor and the administration of oxygen, digitalis and perhaps peripheral vasodilating agents. Recovery from the reaction depends chiefly on the patient's cardiac reserve.

## Use of Tetraethylammonium Bromide as a Diagnostic Test for Pheochromocytoma

John S. LaDue, M.D. *and* (*by invitation*) Paul J. Murison, M.D. *and* George T. Pack, M.D.

*New York, N. Y.*

The pre- and postoperative reactions of a patient with a pheochromocytoma to the intravenous administration of histamine diphosphate and of tetraethylammonium bromide offer a diagnostic test for the presence of epinephrine tumors.

The patient's reactions to intravenous injections of 2 ml. of a saline solution containing 0.025 mg. of histamine phosphate, then of a solution containing 100 mg. of tetraethylammonium bromide and finally of 2 ml. of saline are compared.

Within one minute after the administration of histamine the patient developed a typical attack associated with a rise in blood pressure from 160/105 to 280/160. The reading returned approximately to normal within five minutes. The pulse rate rose from 94 to 116 and then fell to 96. Although the resting blood pressure was somewhat higher before tetraethylammonium bromide was given, the response was just as pronounced and lasted considerably longer. The reading rose from a basal level of 175/105 to 270/160 in thirty seconds and the elevation lasted fifteen minutes. The pulse rate rose from 75 to 130 and returned to 90. The decrease in the blood pressure when the patient changed from a supine to an erect position was dramatic, the reading falling from 230/125 to 95/80. When the 2 ml. injection of saline was given no detectable change in the blood pressure or pulse rate occurred.

The above tests were repeated approximately two months postoperatively and the patient evinced no reaction whatsoever to the injection of histamine, tetraethylammonium bromide or saline.

According to our observations on this patient the use of tetraethylammonium bromide as a test for pheochromocytoma has one advantage over that of histamine. When tetraethylammonium was employed, dangerously high levels of the blood pressure could be controlled simply by having the patient sit up or stand. This resulted in a prompt fall in blood pressure and a disappearance of the symptoms. Lyons and his co-workers noted this phenomenon in their studies on normal and hypertensive individuals; hence, it would appear that with the use of a tilting bed or table tetraethylammonium bromide could be employed with perfect safety in testing for the presence of a pheochromocytoma.

## Peptic Ulcer Therapy—The Use of Synthetic Resins

Manfred Kraemer, M.D.

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In 1945 Segal, Hodge, Watson and Scott reported on the use of a polyamine formaldehyde resin in removing hydrochloric acid from solution. One concludes from this article, that although effective, such large amounts of resin would be needed to inactivate the acid in the stomach that the use of resins in clinical medicine would not be practical. The next year, Martin and Wilkinson found that by using a more finely sieved resin, clinical application might prove practical. One Gm. of their resin,

Amberlite IR 4* took 250 ml. of 0.1 N HCL to pH 4.

We have made neutralization experiments on the freshly extracted gastric juice of one hundred patients. The free acid in these specimens varied from 6 to 69 clinical degrees and in amounts from 15 to 100 ml. Some of the experiments were performed at room temperature and others at 99°F. Toepfer's reagent was used as an indicator. Resin was added with constant stirring. It was found that neutralization took as long as forty-five minutes. Approximately 50 ml. of gastric juice of 25 degrees of acidity was neutralized by 0.1 Gm. of resin. The viscidity of the juice appears to be a factor in determining the number of resin particles exposed to acid.

We have used resin as an antacid in treating forty-seven patients with peptic ulcer in doses of 0.5 to 1 Gm., four to six times a day. We have found it clinically as satisfactory as magnesium trisilicate or aluminum hydroxide and phosphate suspensions. We used it first in powder form but because of its phenolic odor and sandy feeling we found its prescription in capsule form more practical. It appears to have the following advantages over commonly used metallic salt antacids: (1) It has no effect on the acid base balance of the body; (2) it does not alkalinize the urinary tract; (3) it causes neither diarrhea nor constipation; (4) it causes no perianal burning and (5) to date we have noted no toxic or allergic reactions.

## Pharmacological Promotion of Evacuation from the Post-vagotomy Stomach

Stanley H. Lorber, M.D., (*by invitation*) *and* Thomas E. Machella, M.D., *and* (*by invitation*) Horace H. Hodges, M.D.

*Philadelphia, Pa.*

One of the complications that has developed following section of the vagus nerves for peptic ulcer is gastric retention. This occurs especially in those patients who have not had a gastroenterostomy or who do not have an adequately functioning stoma. For relief of the retention some type of a gastroenterostomy has sometimes been required. Such surgical interference, however, has been avoided in six of our postvagotomy patients who had gastric retention by the use of the parasympathomimetic drug, urethane of $\beta$-methyl choline (urecholine).

The drug has been administered orally with each of the main meals of the day in those patients who do not have complete retention. When nothing passes into the intestine the drug must be given subcutaneously. Sublingual or gastric absorption of the drug has not been demonstrated. The usual dose is 5 to 10 mg., but this must be determined for the individual case.

The patients remained free of symptoms of retention while taking the drug but have not done so when a "placebo" was substituted or when the drug was discontinued.

Within five to ten minutes after the subcutaneous injection of a 5 to 10 mg. dose of urecholine, peristaltic activity can be demonstrated roentgenologically or by means of a recording balloon. The period of induced activity lasts forty to sixty minutes and can be reproduced by a second injection. It does not give rise to a significant increase in free hydrochloric acid in the gastric juice when the patient is permitted to swallow saliva or when the stomach contains neutralizing food substances. No untoward side-effects have been noted or complained of when it is administered orally. Following the subcutaneous injection of a 10 mg. dose, flushing, sweating and salivation may occur; sometimes abdominal cramps and a desire to evacuate the urinary bladder also occurs. These phenomena are not so severe as those that follow a comparable dose of mecholyl. The effects of urecholine can be neutralized at any time by an injection of atropine, more promptly when it is given intravenously.

## Effect of Atropine on the Cephalic and Gastric Phases of Gastric Activity

Malcom Block, M.D., W. H. Bachrach, M.D., Joyce Wiltsee Mason., M.D. (*by invitation*) *and* H. M. Pollard, M.D.

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The influence of atropine on the cephalic and gastric phases of gastric activity has never been

*Resinat.

clearly established. Because of the recent introduction of bilateral vagotomy in the treatment of peptic ulceration the effect of atropine on the vagus nerve has become of greater interest in evaluating the importance of these two phases of gastric activity. Adult patients were investigated in this study. Our primary interest was in those with duodenal or gastric ulcers but normal adults were also studied. Gastric motility and secretory activity were studied in the fasting post absorptive period for a continuous period of three to six hours by means of a double lumen tube which was inserted into the stomach. A rubber balloon was attached to the distal tube which was then placed in the antrum. The balloon was inflated with 10 ml. of air and the antral motility recorded on a smoked kymograph through a water manometer. The proximal tube was used for continuous aspiration of gastric contents. All gastric contents passed through a glass electrode attached to a Beckman pH meter so that pH readings could be made at frequent (usually fifteen minute) intervals. Gastric contents were measured for volume, titrated for free and total acidity and analyzed for peptic activity, non-protein nitrogen and protein nitrogen. Atropine was administered subcutaneously and intravenously in doses varying from 0.4 to 1.2 mg. There was some variation in individual cases but generally 0.8 mg. of atropine or more by either route of administration caused a marked decrease in tone and peristaltic type of gastric motility. The volume of gastric secretion was markedly decreased.

The free acidity was generally decreased but there was some variability in this effect. Spontaneous motility and secretory volume could be increased by application of noxious emotional stimuli to the subject. There was a concomitant increase in pulse rate and blood pressure. After atropinization noxious stimuli had no augmenting influence on motility or secretion but other somatic functions responded as indicated by rise in blood pressure and increase in pulse rate. Atropine abolished the increased motor and secretory activity induced by insulin hypoglycemia and had a similar effect in a single case of hypoglycemia. Atropine given after the administration of liver extract by the stomach tube decreased the motor activity of the stomach but had little or no influence on secretory activity.

## Aqueous Suspensions of Crystalline Estrogenic Substances. A Comparative Assay

Allan C. Barnes, M.D. *and* (*by invitation*) William Cope, M.D.

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Since the introduction by Freed and Greenhill of aqueous suspensions of crystalline estrogens this form of therapy has received increasing attention. In comparison with pellet implantation, injections of such suspensions are simpler and introduce the material in more finely divided form. In contrast with oil and wax solutions there is no sensitivity to the vehicle and no formation of encapsulated collections of foreign matter.

To date the reported assays of crystalline estrogens in aqueous suspension have been based on the subjective response of menopausal women and the original comparison was with a crystalline suspension—not solution—in oil. The present study was undertaken to provide more objective evidence by a comparative assay in humans of equivalent therapeutic products.

The preparations under study were administered to a group of post-menopausal women and the response of the vaginal epithelium was followed by vaginal smears. These were stained by the Shorr technic and were classified in three groups: 1. Atrophic. There was complete or almost complete absence of estrogenic stimulation. The smear had a high white count, small cells and vesicular nuclei. The stain was predominantly blue; 2. intermediate. There was some estrogenic stimulation with few white cells, pyknotic as well as vesicular nuclei and the stain was predominantly green. 3. advanced. There was full stimulation with large, flat cells with pyknotic nuclei. The stain was predominantly red.

A suspension of estrogenic substance in water was compared by this method with a solution of estrogenic substance in oil and with solutions of alpha-estradiol in oil. The highest levels were

invariable reached with the aqueous suspension and were usually found on the fifth or sixth day.

The duration of effect of the aqueous suspension was greater than a comparable amount of estrogenic substance in oil, as well as a comparable unitage of the estradiol. Doubling the dose of either form of medication had a tendency to double the effective time without necessarily raising the peak level of reaction. The response to a 2 mg. dose of any of the drugs under consideration was limited to two weeks. There was no evidence of a prolonged action (up to ten weeks) reported on the basis of subjective response.

## Experience with the Thymol Turbidity Test on a General Medical Service

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(Introduced by Bruce Logue, M.D.)

The thymol turbidity test was performed on the sera of approximately 500 patients from an active medical service. Many of these patients were followed serially during and after their hospital stay. The cases included a number of patients in whom liver dysfunction was not suspected.

The thymol turbidity test was found to be an excellent index of liver function in cases of infectious hepatitis and cirrhosis. The results confirmed previous work done with these diseases. The test is of great value in following the progress of cases of infectious hepatitis.

A series of twenty-one proven cases of lymphogranuloma venereum was studied and twenty of them were found to have positive thymol turbidity tests. In view of this the positive thymol turbidity test must be treated with caution in negro patients. The test was also frequently positive in patients with rheumatoid arthritis, congestive failure and strongly positive in such generalized diseases as disseminated lupus erythematosus, dermatomyositis and scleroderma.

The thymol turbidity test was compared to the cephalin flocculation test and it was found that both tests were positive in 35 per cent of the cases, both negative in 37 per cent; thymol turbidity positive and cephalin flocculation negative in 22 per cent while the thymol turbidity was negative and the cephalin flocculation positive in 6 per cent of the cases.

## Enhancement of Plasma Penicillin Concentrations by Caronamide and Sodium Benzoate

Elias Strauss, M.D.

From the Departments of Medicine and Bacteriology, Southwestern Medical College, Dallas, Tex.

Caronamide (4- carboxy phenylmethane sulfonanilide) has been found to decrease the renal excretion of penicillin. In clinical trials the oral administration of caronamide increased plasma penicillin concentrations when penicillin was administered intramuscularly as well as orally.

In the present study the effect of caronamide on plasma penicillin concentrations was determined in approximately fifty normal adults. Penicillin was administered by various routes during the day and plasma concentrations determined. On a succeeding day the same subjects received penicillin on the same dosage schedule and in addition, took caronamide by mouth, usually in a dosage of 2 Gm. every two hours. No evidences of toxicity clearly attributable to caronamide were encountered.

The administration of 100,000 units of penicillin by mouth every two hours resulted in low plasma concentrations one and two hours after each dose. The concurrent administration of caronamide resulted in a two to sixteen-fold increase in plasma penicillin concentrations in a great majority of instances.

In subjects who received 25,000 and 50,000 units of penicillin intramuscularly the concurrent administration of caronamide likewise resulted in a two to eight-fold increase in plasma penicillin concentrations in most instances. The effect of caronamide in a fixed dosage was more marked with smaller than with larger doses of penicillin. Some data on the rapidity and duration of the action of caronamide were obtained.

When penicillin was administered in beeswax and peanut oil the effects of caronamide were variable but in the majority of instances plasma penicillin concentrations were increased.

Caronamide had no effect on the plasma concentration of streptomycin when the latter

was administered in multiple intramuscular injections.

Sodium benzoate, in general, was as effective as caronamide in increasing plasma penicillin concentrations when penicillin was administered by mouth or intramuscularly. The administration of both sodium benzoate and caronamide to subjects receiving penicillin by mouth resulted in a greater increase in plasma penicillin concentrations than was affected by either of these agents alone.

## A Study of the Analgesic and Toxic Properties of Dolophine (6-Dimethyl-amino-4, 4-Diphenyl 3-Heptanone Hydrochloride). A Preliminary Report

Donald I. Bryan, M.D. *(by invitation)* and Charley J. Smyth, M.D.

From the Department of Medicine of Wayne University, Detroit, and the Wayne County General Hospital, Eloise, Mich.

A study of the analgesic and toxic properties of 6-dimethyl-amino-4, 4 diphenyl 3-heptanone hydrochloride, also known as dolophine or #10820, has been made in ninety-four patients.

Pain in the majority of these patients was due to advanced malignant disease. In the remainder of the group the effect of this drug on the pain associated with dysmenorrhea, osteomyelitis, rheumatoid arthritis, peptic ulcer and panophthalmitis was studied. A small group was studied for suppression of cough and in a series of five patients the drug was used for pre- and postoperative analgesia.

Ages of the patients studied ranged from fourteen to eighty-eight years; the largest number of patients were from forty-five to sixty-five years of age. Oral and subcutaneous routes of administration were used. According to the degree of pain single subcutaneous dosages were varied from 2.5 mg. to 17.5 mg. The single oral dose varied from 2.5 mg. to a maximum of 10 mg. A total of approximately 10,000 injections and approximately 500 oral doses have been given.

Analgesia was obtained in seventy-two patients to whom the drug was given subcutaneously and it was maintained at satisfactory levels in all but a small group. Duration of pain relief varied from two to eight hours; in most patients relief was sustained for four hours. In twenty-one patients who were given the drug orally the most satisfactory results were obtained in cough suppression.

Toxic reactions occurred in only five of the seventy-two patients given the drug subcutaneously. These reactions were a slight lethargy in two, apprehension and paresthesias in one, severe delirium in one and transitory hallucinations in another. Toxic manifestations were common in the patients who received the drug by mouth. Of twenty-one patients, sixteen had one or more of the following reactions: anorexia, nausea, vomiting, diarrhea, dizziness, weakness or diaphoresis.

These early clinical experiences indicate that dolophine is a powerful analgesic agent which may be used subcutaneously with a minimum of toxic reactions. Insufficient data is available to determine the tolerance and habituation factors.

The work was supported by a grant from the Eli Lilly Company, Indianapolis, Ind.

## Pharmacology and the Clinical Use of Dolophine (6-Dimethyl-amino-4, 4-Diphenyl 3-Heptanone Hydrochloride)

K. G. Kohlstaedt, M.D. *and (by invitation)* Paul Clouse, M.D., W. V. Lee, M.D. *and* C. C. Scott, M.D.

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind.

The analgesic effects of dolophine have been studied—men and animals serving as test subjects. In rats the intraperitoneal injection of 1 to 2 mg. per Kg. produced analgesia as measured by the tail-pinching method of Haffner. Using the Hardy, Wolff, Goodell thermal technic, dolophine (5 mg. per Kg.) was administered subcutaneously to dogs and was found to be more potent than the same amount of morphine. Five mg. raised the pain threshold in man.

More than 300 patients have received dolophine for the relief of pain (2.5 to 15 mg. subcutaneously or orally). Analgesia was main-

tained at a satisfactory level in 79 per cent of these individuals. On the general and gynecological surgery wards postoperative pain and discomfort were completely controlled in sixty-four out of seventy-nine patients. The administration of dolophine at regular intervals alleviated severe, chronic pain for a period of six months in ten patients.

This heptanone derivative in doses of 1.5 to 2.0 mg. (by mouth) was found to suppress the cough reflex. It has been used for this purpose in patients with bronchiectasis, pertussis, bronchiogenic carcinoma and chronic passive pulmonary congestion due to cardiac failure. In forty-eight patients with advanced pulmonary tuberculosis, pleuritic pain and cough were controlled as well or better with dolophine than with codeine. The effect of therapeutic doses of dolophine on respiration, the electrocardiogram, rectal temperature, arterial pressure and hepatic function has been observed. This compound occasionally caused unpleasant side reaction, e.g., nausea, vomiting, light-headedness, diaphoresis and mental confusion. These untoward manifestations occurred following both oral and parenteral administration and were most frequent in ambulatory patients with mild pain.

Analgesic doses of this drug produced less sedation and narcosis than therapeutically equivalent amounts of morphine. The results of clinical studies indicate that dolophine is a potent analgesic and that it may be used to alleviate many types of pain.

## Clinical Observations in Patients Treated with Antireticular Cytotoxic Serum. Preliminary Report

W. D. Davis, Jr., M.D.

From the Department of Medicine, Tulane University School of Medicine and the Department of Medicine, Ochsner Clinic, New Orleans, La.

Within the past eighteen months 106 patients have been treated with antireticular cytotoxic serum in the Ochsner Clinic. Material was prepared by Dr. Harry Goldblatt according to the method of Marchuk and was usually administered subcutaneously in dosages of 0.5 ml. of 1 to 10 dilution on the first day, 1.0 ml. on the fourth day and 1.5 ml. on the seventh day; courses were generally repeated every six weeks.

The series here reported consists of sixty-two cases of far advanced cancer and thirty-eight cases of other diseases. Of the sixty-two patients with cancer, forty-nine are dead and thirteen living, the longest length of life being thirteen months from the first injection of serum. Although it cannot be said that any lives were prolonged by this serum treatment, ten patients experienced a marked relief of pain, a gain in weight, an increased appetite, a sense of well-being and eleven noted moderate benefit.

The second group of thirty-eight cases includes twenty-one cases of rheumatoid arthritis; three of degenerative arthritis; two each of osteoporosis, Hodgkin's disease and acute monocytic leukemia; single cases of myeloid leukemia, gouty arthritis, lupus erythematosus disseminatus, radiation neuritis, dermatomyositis, multiple myeloma, senile emphysema and epidural abscess. Pronounced relief was obtained by twelve patients with rheumatoid arthritis and moderate relief by one. The patient with myeloid leukemia responded dramatically to repeated injections. The patients with osteoporosis had complete relief of pain and those with Hodgkin's disease, multiple myeloma and lupus erythematosus disseminatus have obtained moderate benefit; the others have not been helped at all. Both patients with acute monocytic leukemia and the one with multiple myeloma are now dead.

Reactions occurred in twelve patients, nine of whom were in the non-cancerous group. These usually consisted of indurated, reddened, painful areas of varying extent in the injected arm, plus a fever of about 101°F. However, rash, arthralgia and bleb formation occurred in a few instances.

Particularly interesting cases, reported in detail, include: (1) a patient with carcinoma of the cervix in whom symptoms have completely disappeared following five courses of injections; (2) the patient with myeloid leukemia whose peripheral blood made repeated responses to the course of serum and (3) cases of rheumatoid arthritis and osteoporosis in which dramatic relief of pain has been noted.

## Intravenous Use of Sodium Amytal in Psychosomatic Disorders

Herbert S. Ripley, M.D. *and* Stewart Wolf, M.D.

From the New York Hospital and the Departments of Medicine and Psychiatry, Cornell University Medical College, New York, N. Y.

The action of intravenously administered sodium amytal in 500 patients in military and civilian practice has been analyzed from the standpoints of its usefulness in diagnosis, treatment, prognosis and investigation of the etiology of bodily disorders arising from problems of personality adjustment.

The drug has been found useful, chiefly as follows:

1. Diagnostically in distinguishing between irreversible, structurally determined disorders and functional disorders of organ systems.
2. Diagnostically in distinguishing between neurosis and malingering.
3. Diagnostically in the elucidation of dynamically significant situational conflicts.
4. Therapeutically in the alleviation of troublesome symptoms.
5. Therapeutically when data obtained during sodium amytal interview are used in formulation to the patient, when the reassuring value of the reversibility of symptoms is used or in the use of hypnotic or posthypnotic suggestions.
6. Prognostically in determining the depth of the disturbance and its susceptibility to treatment.
7. From an investigational standpoint in rendering modifiable the bodily disturbances of various diseases.

The most suitable subjects for narcoanalysis under sodium amytal are those with disorders of personality adjustment of a relatively short duration. The drug is less useful in diagnosis or treatment of patients with rigid personalities or a long standing pattern of disability.

Sodium amytal is a highly useful tool in medicine but it is in no sense a specific or automatic agent. It is only of substantial value in the hands of a skillful physician who takes appropriate advantage of the state induced to gain diagnostic or therapeutic leverage.

## Further Observations on the Action of Dibenamine in Human Subjects

H. H. Hecht, M.D., *and* (*by invitation*) Ferne S. Focht, M.D., J. A. Abildskov, M.D., T. W. Burns, M.D. *and* R. O. Christensen, M.D.

From the Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah.

Dibenzyl-$\beta$-chlorethyl amine (dibenamine), a compound related to the nitrogen mustards, has been claimed to possess certain sympatholytic properties in animals. In view of the clinical usefulness that an effective blocking agent for the adrenergic system would have, a study of the effect of the administration of dibenamine hydrochloride in seventy patients was made.

Only the intravenous route was found to yield consistent results. Not more than 4 to 6 mg./Kg. body weight were tolerated and transient toxic reactions occurred in almost all individuals. The most remarkable of these was a psychic disturbance consisting of repetitive preceptions of actual events and hallucinatory episodes which were associated with a disturbance of time sensations. Consciousness usually remained unimpaired and full insight into the psychotic state was present during the reaction. This bizarre alteration of mental function and of thought processes was noted in fifteen patients (21 per cent) but may have gone unnoticed in others.

The psychosis lasted for two to three hours while the height of the pharmacological action of dibenamine extended at least over a twenty-four-hour period. In some patients the response to standard sympathetic stimuli was altered for several days following a single injection.

After the administration of dibenamine it was seen that some of the excitatory effects of sympathin E released by stimulation of the adrenergic system were altered or appeared completely blocked when tested in the resting patient following standard exercises or upon the intravenous administration of sympathomimetic compounds. Some of the expected responses to parenterally administered epinephrine remained unchanged or appeared potentiated by dibenamine.

The actions of the compound appear to be specifically directed toward certain excitatory effects of epinephrine but dibenamine cannot be

regarded as a neutralizer of the sympathetic nervous system in all respects. In the doses that can be safely administered to man dibenamine is regarded as a valuable pharmacological tool but appears to have limited therapeutic possibilities.

## Extent of Vasodilatation Induced in Different Vascular Beds after Systemic Autonomic Blockade with Tetraethylammonium

S. W. Hoobler, M.D., Rosalie B. Neligh, M.D., Gordon K. Moe, Ph. D., S. Don Malton, M.D., Saul Cohen, Ph. D., H. T. Ballantine, Jr., M.D., (*by invitation*) *and* R. H. Lyons, M.D.

From the Departments of Medicine, Surgery and Pharmacology, University of Michigan Medical School, Ann Arbor, Mich.

In normotensive and hypertensive subjects injection of tetraethylammonium results in a temporary decrease in the total peripheral resistance as manifested by a reduction in mean arterial pressure and a maintained or increased cardiac output. The blood flow through vascular beds believed to be normally under some degree of vasomotor tone is markedly increased. The volume flow of blood in the hands and feet, as measured by the venous occlusion plethysmograph, increases significantly above resting levels following the administration of 500 mg. of the drug; it exceeds the levels achieved by other vasodilating drugs and may occur despite a marked decrease in arterial pressure. Blood flow seldom reaches the levels seen after sympathetic block with metycaine or after heat to the trunk suggesting that a complete autonomic blockade is not produced. On the other hand there is a rise in cutaneous circulation as judged by an increase in skin temperature of the hands and feet frequently with abolition of the temperature gradient. After sympathetic denervation of the extremity the blood flow is not further increased by administration of this drug. Induced vasoconstrictor reflexes in the hands and feet are reduced or abolished after tetraethylammonium. Blood flow in the forearm is only slightly increased after administration of the drug. Renal blood flow as measured by para-amino hippurate clearance is unaffected despite variable reductions in arterial pressure in hypertensive and normal subjects. It is suggested that an increase in the blood flow through various vascular beds following tetraethylammonium depends on the presence of a neurogenic vasoconstrictor tone in these areas.

## Mechanism of the Fall in Arterial Pressure Produced by High Spinal Anesthesia in Patients with Essential Hypertension

William C. Levin, M.D. *and* (*by invitation*) Raymond Gregory, M.D.

*Galveston, Texas*

The problem has been studied by the simultaneous recording of arterial and venous pressures before and during high spinal anesthesia in five patients with normal blood pressures and in ten patients with essential hypertension.

The results demonstrate no constant correlation of changes in venous and arterial pressures. Arterial pressure may fall without any significant fall in venous pressure or may be preceded or followed by a fall in venous pressure. A fall in venous pressure may also occur without any significant fall in arterial pressure.

The conclusion is drawn that there is no cause and effect relationship for the decreases in arterial and venous pressure which may follow induction of high spinal anesthesia in patients with essential hypertension. It is further concluded that the fall in arterial pressure in such patients during high spinal anesthesia is probably not due to decreased cardiac output produced by diminished venous return.

## Criteria for the Diagnosis of Right Ventricular Hypertrophy Using Unipolar Limb and Precordial Leads

Maurice Sokolow, M.D. *and* (*by invitation*) Thomas P. Lyon, M.D.

From the Division of Medicine, University of California Medical School, San Francisco, Calif.

The roentgen diagnosis of right ventricular hypertrophy rests on less secure grounds than does that of left ventricular hypertrophy; therefore, other methods of diagnosis become of con-

siderable importance. The use of unipolar limb and precordial leads has provided a valuable means of recognizing right ventricular hypertrophy. With the present surgical approach to congenital pulmonary stenosis, the differentiation of this condition from other cyanotic congenital cardiac defects is especially important. Right ventricular hypertrophy is almost universally present in the tetralogy of Fallot, whereas early in the course of other lesions, such as patent interauricular septal defects, right ventricular hypertrophy may be absent. In patients with chronic pulmonary disease such as emphysema, pulmonary fibrosis, etc., it is often difficult to determine whether the condition is entirely pulmonary or whether chronic cor pulmonale has been superimposed. In this group of cases unipolar leads are of definite value.

Fifty cases of right ventricular hypertrophy have been studied with standard and unipolar leads and in many with further exploratory unipolar leads over the right anterior chest and abdomen. The patients with tetralogy of Fallot presented the most typical and complete pattern of right ventricular hypertrophy, whereas those with chronic cor pulmonale frequently had some of the typical electrocardiographic features absent, especially those from the right precordium.

The characteristic combination of findings in right ventricular hypertrophy include:

1. In leads from the right precordium and xiphoid, (1) a prominent, often tall R wave, (2) a small, often absent S wave, (3) a delayed onset of the intrinsic deflection (more than .03 second), (4) a depressed S-T segment and an inverted T wave, (5) an abnormally great ratio of the amplitude of R/S;

2. in leads from the left precordium, (1) a small R wave, (2) a deep S wave, usually greater than the R wave in the same lead, (3) a normal or shortened onset of the intrinsic deflection;

3. in the unipolar limb leads, (1) a prominent R wave in $aV_R$, (2) abnormal T waves in $aV_F$ or $aV_L$ depending on the position of the heart and (3) prominent P waves in $aV_F$.

The unipolar limb leads, in addition, were valuable in differentiating right axis deviation (as seen in the standard limb leads) due to a vertically placed heart as the result of right ventricular hypertrophy. In some patients with chronic pulmonary emphysema or mitral stenosis the unipolar leads demonstrated that right axis deviation of +90 to 105° was due to a vertical heart and not to right ventricular hypertrophy. In others with similar standard leads right ventricular hypertrophy was demonstrated.

The importance of lead $V_1$ will be discussed since this lead frequently demonstrates the major abnormality. When right bundle branch is associated with right ventricular hypertrophy the abnormality may be seen in $V_1$ and not in $V_2$.

## Electrocardiograms with Poor Prognosis in Acute Coronary Occlusion

Richard Gubner, M.D. *and* Alexander I. Thomashow, M.D.

From the Department of Medicine, Long Island College of Medicine, Brooklyn, N. Y.

This study comprises an analysis of the electrocardiographic findings in one hundred first attacks of acute myocardial infarction in subjects without antecedent heart disease. Comparison was made between fifty cases who succumbed within a period of six weeks and fifty cases who survived the attack.

Certain electrocardiographic features were found to occur preponderantly in the fatal cases and relatively infrequently in the surviving cases. In anterior infarction these included deep Q wave in lead I, the ABB pattern and bundle branch block. A review of cases reported in the literature indicates that these electrocardiographic changes are very commonly observed in ventricular aneurysm which is a sequel to extensive anterior infarction. When such changes occurred in surviving cases of anterior infarction they were usually transitory. Fatal cases of posterior infarction exhibited a much greater frequency of marked S-T segmental deviation, particularly marked depression in the precordial lead, bundle branch block and T changes in leads I and IV in addition to the usual $Q_2$, $Q_3$, $T_2$, $T_3$ pattern.

In addition to these changes which appear to

indicate extensive infarction other findings occurred preponderantly in the fatal group. Among these were: electrocardiographic characteristics of combined anterior and posterior infarction, progressive changes other than the usual serial S-T and T evolution, electrical alternans, very low voltage of the QRS complex, prolongation of the Q-T interval, depression of the P-R interval and P changes suggesting associated auricular infarction, major arrhythmias such as auricular fibrillation, ventricular tachycardia and heart block and sinus tachycardia exceeding 110. Mention is made of other electrocardiographic findings which have been found to accentuate the gravity of acute myocardial infarction, such as Q and T changes in multiple chest leads and high P waves which appear to be associated with acute cardiac decompensation.

Among fatal cases of acute myocardial infarction over 80 per cent exhibited one or more of the findings described while in surviving cases the majority presented the simple anterior or posterior pattern. No significant difference was found in the incidence or mortality of anterior and posterior infarction *per se*.

## Relative Effectiveness of Various Diuretics in Causing Sodium Excretion in Pregnant Women

Willis E. Brown, M.D., *and*
J. T. Bradbury, Sc.D.

From the Department of Obstetrics and Gynecology, University of Iowa, Iowa City, Ia.

Patients in the last trimester of pregnancy have been placed on diets which furnished a constant intake of sodium and twenty-four-hour urine collections have been made for several weeks. After adequate periods of control observations, diuretic agents were administered and their effect of urine volume and sodium elimination were determined.

Mercurial diuretics (mercuhydrin and salyrgan) cause a marked loss of sodium chloride. This is accomplished by maintaining a relatively high concentration of sodium in the urine together with an increased urine volume. The sodium depletion after the administration of a mercurial drug may be so great that dietary sodium is retained for a day or two in order to replenish the body stores.

Ammonium chloride in doses of 8 to 16 Gm. per day causes an acidosis in which sodium is eliminated in the urine. The initial effects may be more marked than those obtained on the second and third days so that prolonged treatment is relatively ineffective in causing a continuous loss of sodium. Urine volumes are not consistently increased.

Two hundred grams of glucose was administered intravenously in volumes ranging from 400 ml. to 4,000 ml. The effect on urinary volume is directly related to the volume of solution injected rather than to the concentration of the glucose. In no instance was the urinary volume increased sufficiently to account for all of the administered fluid. With increased urine volume there is a marked drop in sodium concentration so that no increase in sodium excretion could be demonstrated.

Aminophylline in large doses (7.5 gr. three times a day) has an effect similar to that of the mercurials, in that relatively high concentrations of sodium are maintained in the urine during the periods of increased volume output.

In two patients with toxemia of pregnancy, intravenous glucose solutions did not mobilize tissue fluids or sodium, whereas there was a marked increase in urine volume, sodium excretion and a loss of weight when they were given a mercurial drug. One eclamptic patient was given aminophylline after a six hour interval of anuria; a progressive diuresis started within one hour and the urine contained high concentrations of sodium.

## Function of the Stomach as Observed in Fistulous Human Subjects, with Special Reference to the Action of Drugs and the Effects of Vagotomy

Stewart Wolf, M.D. *and*
Harold G. Wolff, M.D.

*New York, N. Y.*

A human subject with a gastric fistula larger than that of Alexis St. Martin was studied in detail continuously for over five years. The

effects on the stomach of a large number of drugs and chemical agents were determined on this subject as well as on two other fistulous individuals. One of the latter was studied before and after bilateral supradiaphragmatic vagotomy.

The data obtained fell into five categories, allowing of the following inferences:

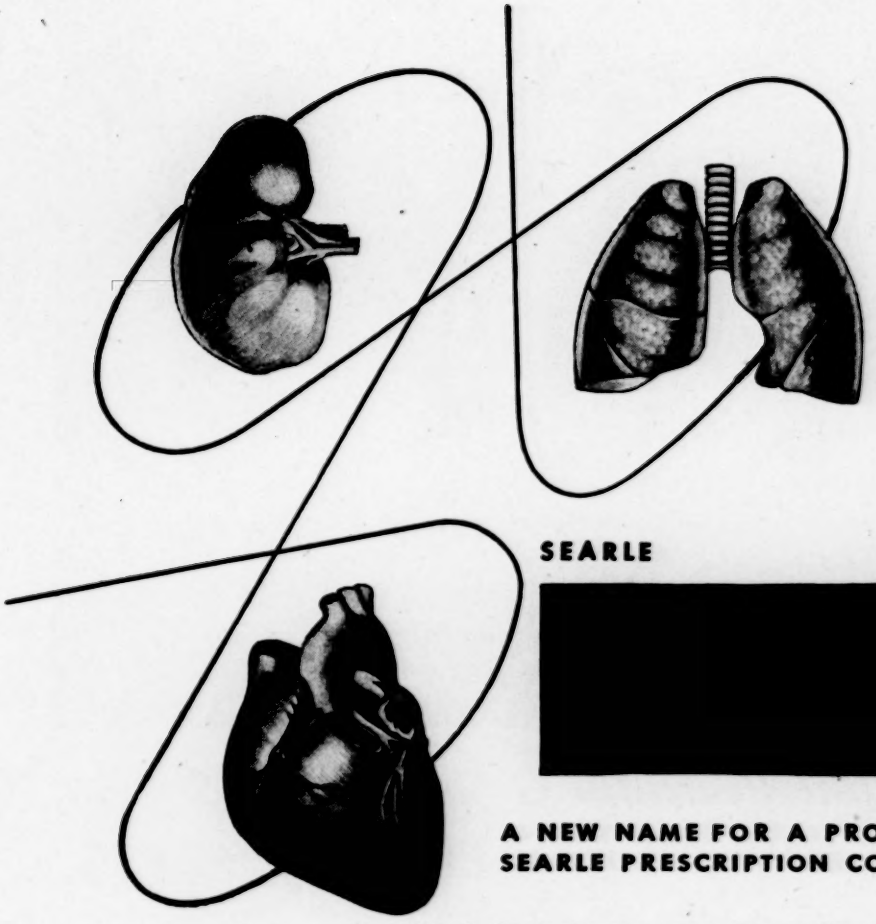
1. Secretory and motor activity in the stomach usually parallel one another and these gastric functions correspond closely to the blood flow through the organ. Drugs which inhibit gastric function induce a state of pallor and deturgescence in the stomach; drugs which stimulate gastric activity on the other hand give rise to hyperemia and turgidity of the membrane.

2. The gastric mucous membrane is remarkably resistant to trauma during pallor and relative inactivity of the stomach. With hyperemia and engorgement, however, the membrane becomes far more vulnerable to physical insult, erosions and bleeding points resulting readily from minor traumas. When hyperemia and engorgement are intense and sustained a lowering of the pain threshold occurs so that ordinarily painless gastric contractions become painful.

3. By virtue of its protective covering of mucus the gastric mucosa, even when red and turgid, is comparatively resistant to the action of caustic chemical agents. It was found that various drugs, including emetics commonly thought to be gastric irritants, actually do not exert an irritant effect on the stomach. Locally acting emetics exert their effects after passage into the duodenum.

4. Following vagotomy the stomach remained pale and inactive for several weeks. Slight hyperemia followed the ingestion of food but situational stimuli provocative of conflict with anger and resentment, accompanied by intense gastric hyperemia before operation failed to induce such a change after the vagus innervation had been interrupted.

5. In general, the effects on the stomach of a given quantity of any drug could not be predicted without reference to the prevailing state of the organ. Gastric inhibitors, for example, whose effects were readily demonstrable when the stomach was in an average state of engorgement and activity exerted no detectable effect when the stomach was turgid and over-active. Situational stimuli provocative of emotional changes were found to be of great importance in determining the state of the stomach. Profound alterations in gastric function associated with troublesome symptoms repeatedly followed the administration of placebos. Thus, the actions of the various drugs tested depended in large measure upon whether they reinforced or opposed the other influences acting at the same time.



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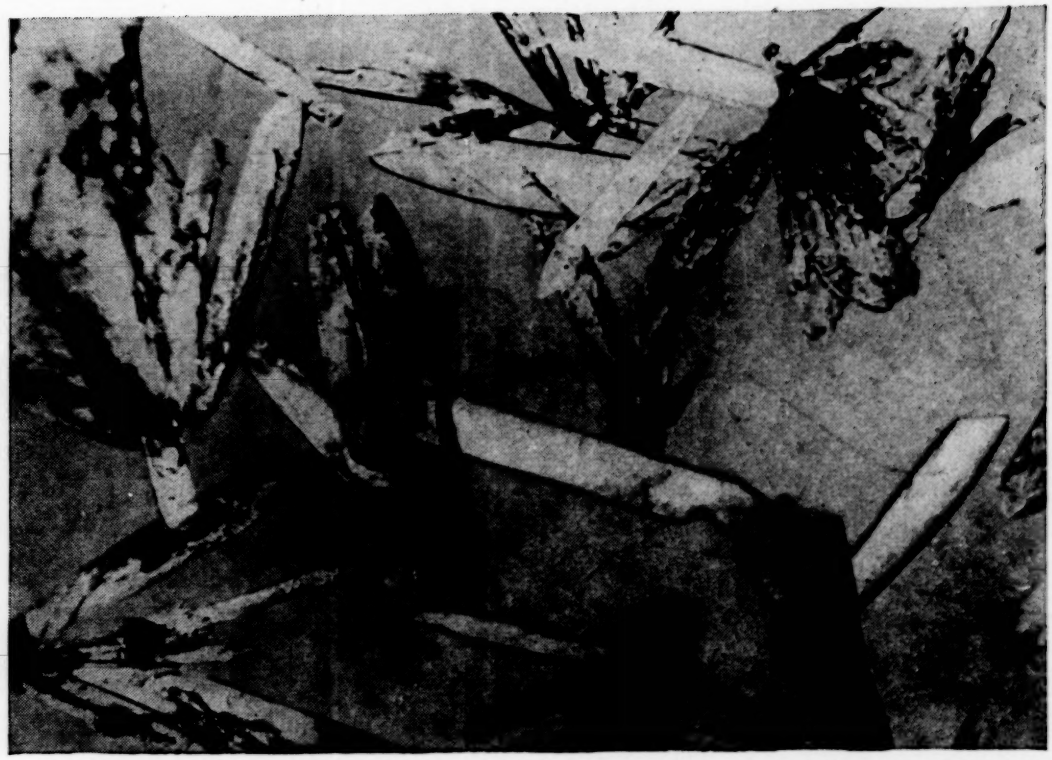

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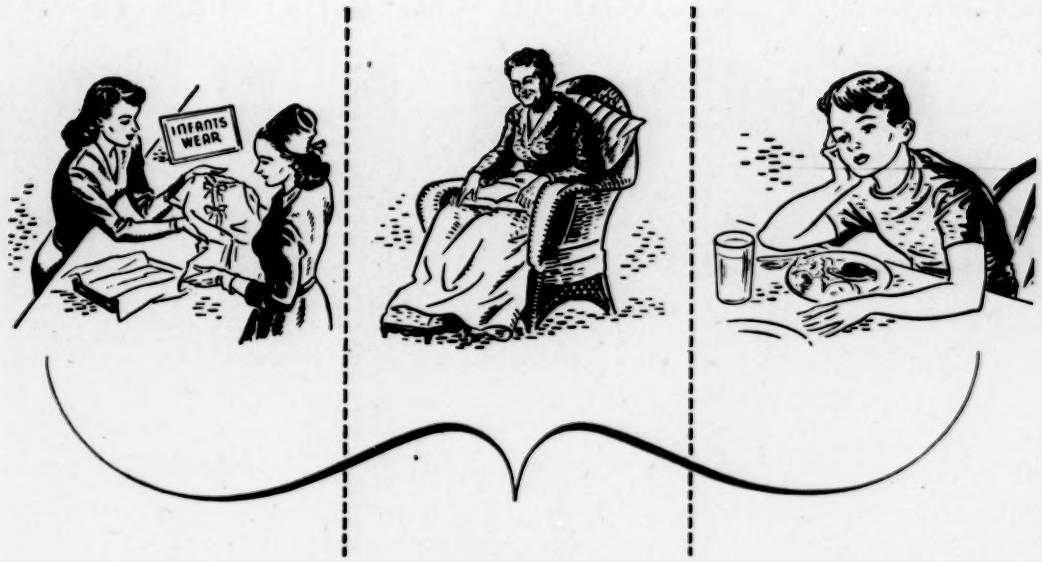


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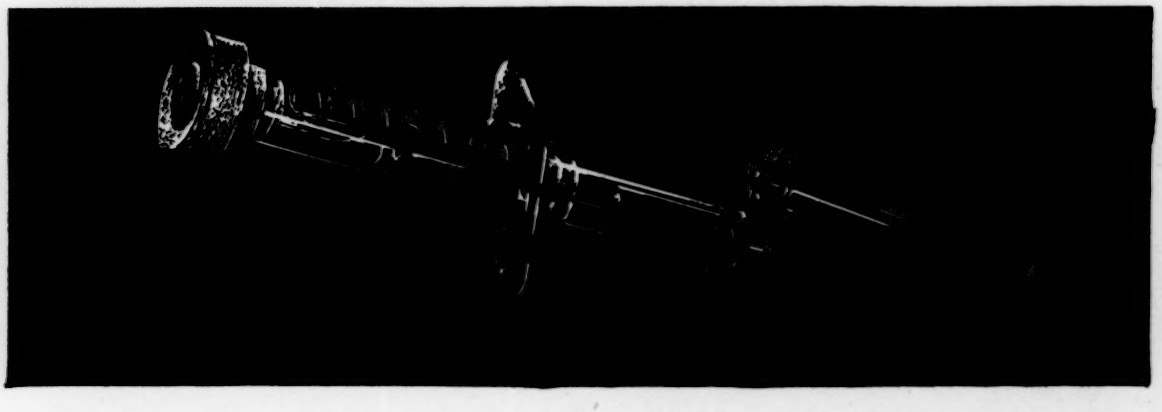

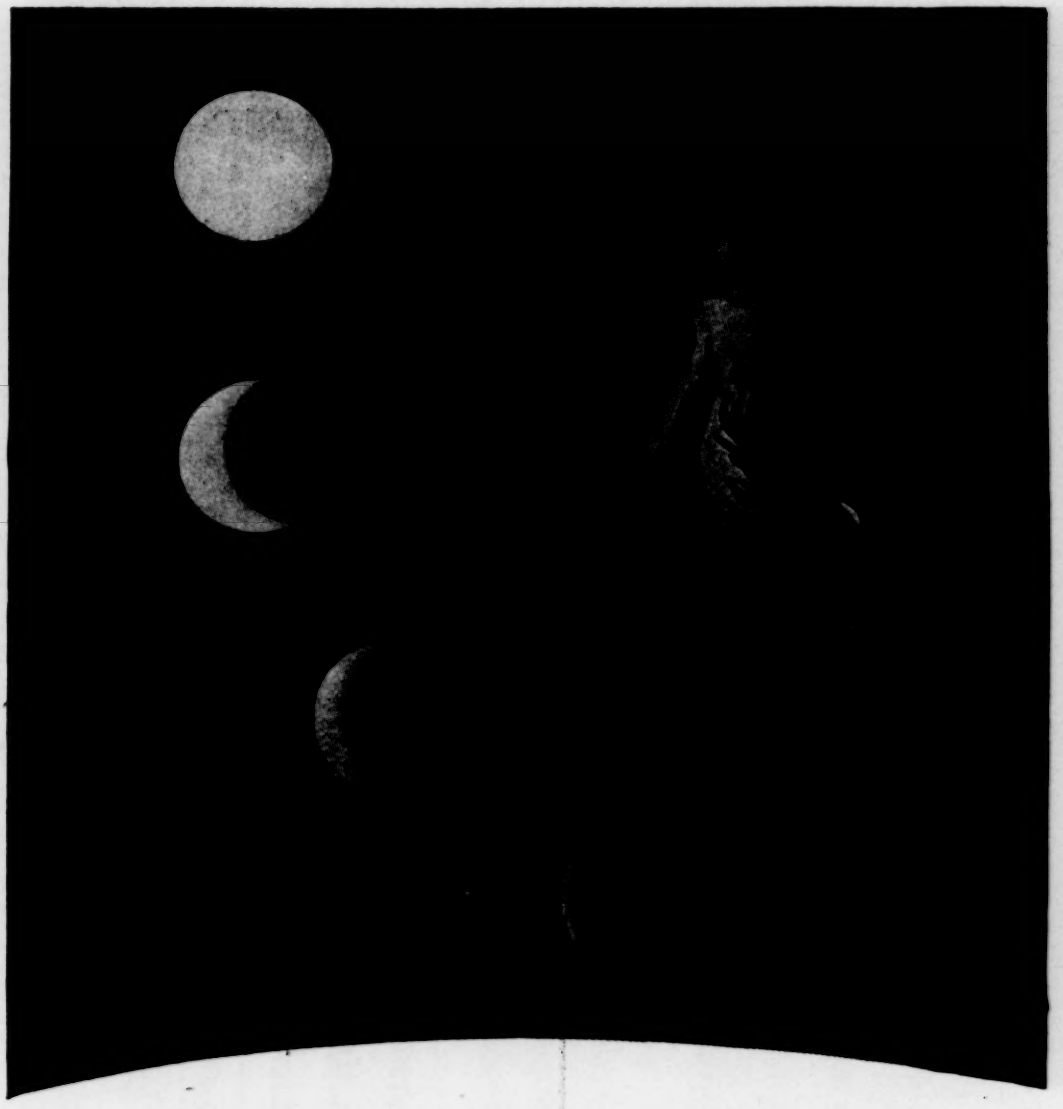

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DI-OVOCYLIN (brand of α-estradiol dipropionate). T. M. Reg. U. S. Pat. Off. and Canada.

For further information, write Professional Service Department.

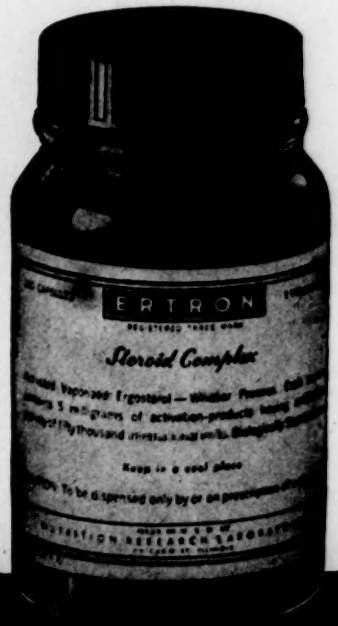
CIBA PHARMACEUTICAL PRODUCTS, INC. SUMMIT, NEW JERSEY

# ARTHRITIS IS DIFFICULT TO DIAGNOSE

*Many symptoms of Arthritis are so confusing that they make the Physician's diagnosis extremely difficult. One compensation is that when the diagnosis indicates chronic Arthritis, there is available Ertron—Steroid Complex, Whittier . . . more widely prescribed than any other antiarthritic. Ertron is safe.*

# ERTRON

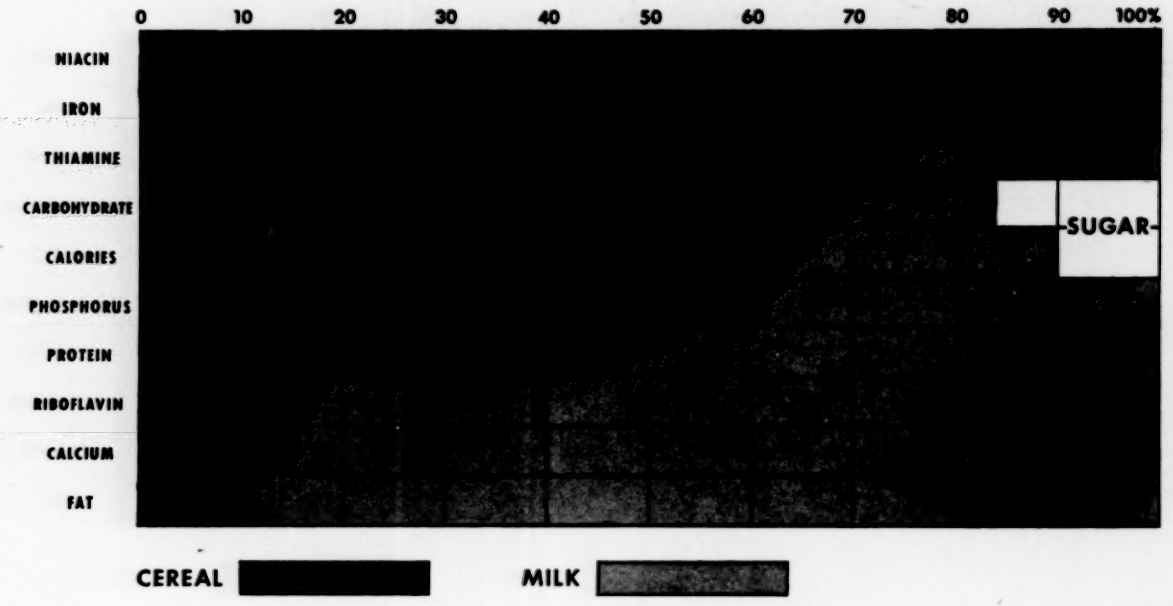
**Steroid Complex, Whittier**




NUTRITION RESEARCH LABORATORIES

CHICAGO

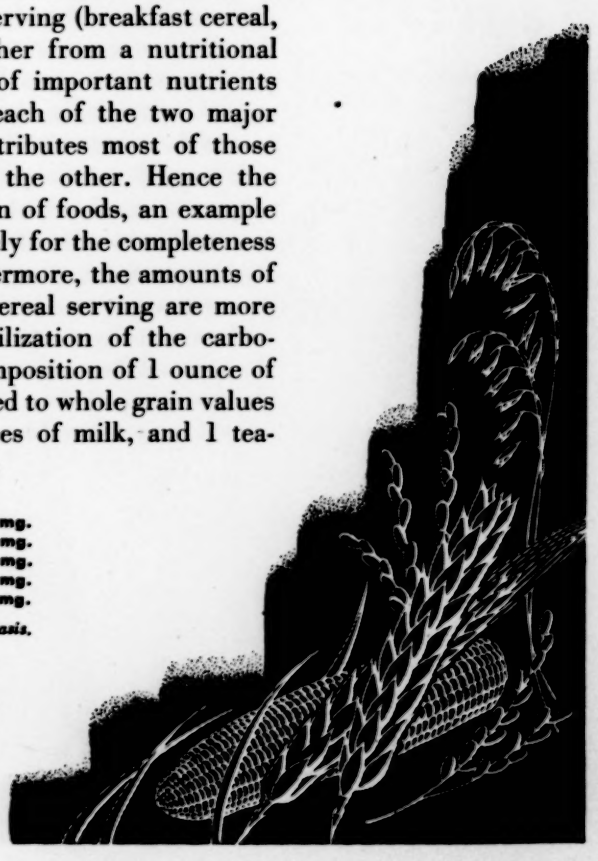
# Excellent Nutritional Supplementation




The chart reproduced above illustrates the excellent manner in which the foods comprising the cereal serving (breakfast cereal, milk, and sugar) complement each other from a nutritional standpoint. It depicts the percentage of important nutrients contributed by each food. Note that each of the two major components of the cereal serving contributes most of those nutrients supplied less generously by the other. Hence the cereal serving is a balanced combination of foods, an example of the importance of selecting foods wisely for the completeness of their combined contributions. Furthermore, the amounts of B-complex vitamins contained in the cereal serving are more than adequate to permit metabolic utilization of the carbohydrates provided. The quantitative composition of 1 ounce of cereal* (whole grain, enriched, or restored to whole grain values of thiamine, niacin, and iron), 4 ounces of milk, and 1 teaspoonful of sugar is shown in the table.

| | | | |
|---|---|---|---|
| CALORIES..... | 202 | PHOSPHORUS... | 206 mg. |
| PROTEIN..... | 7.1 Gm. | IRON..... | 1.6 mg. |
| FAT..... | 5.0 Gm. | THIAMINE..... | 0.17 mg. |
| CARBOHYDRATE | 33.0 Gm. | RIBOFLAVIN.... | 0.24 mg. |
| CALCIUM..... | 156 mg. | NIACIN..... | 1.4 mg. |

**Composite average of all breakfast cereals on dry weight basis.*



**CEREAL INSTITUTE, INC.**

135 South La Salle Street • Chicago 3

*The presence of this seal indicates that all nutritional statements in this advertisement have been found acceptable by the Council on Foods and Nutrition of the American Medical Association.*

*Indispensable for reproduction*

# PRANONE
# PROLUTON

Schering

# What every bride *shouldn't* know:

WHAT it feels like to be poor . . .

What it feels like when your first-born needs an expensive doctor—and you can't afford it . . .

What it's like wanting a home of your own . . . and never quite getting it . . .

What it's like having your kids grow up not knowing whether they'll ever get to college . . .

What it's like to see the Joneses and the Does and the Smiths able to travel abroad—but never you . . .

What it's like to have to keep telling yourself, "He may not have money, but he's my Joe."

There is no cure-all for all these things.

But the closest thing to it for most of us is buying U.S. Savings Bonds—automatically. So here's a bit of friendly advice for newlyweds:

**Get on the Payroll Savings Plan** where you work or the Bond-a-Month Plan where you have a checking account.

Either plan helps you save money regularly, automatically, and *surely*, for the things you want.

It's one of the finest things you can do to start married life right.

## Save the easy, automatic way...with U.S. Savings Bonds

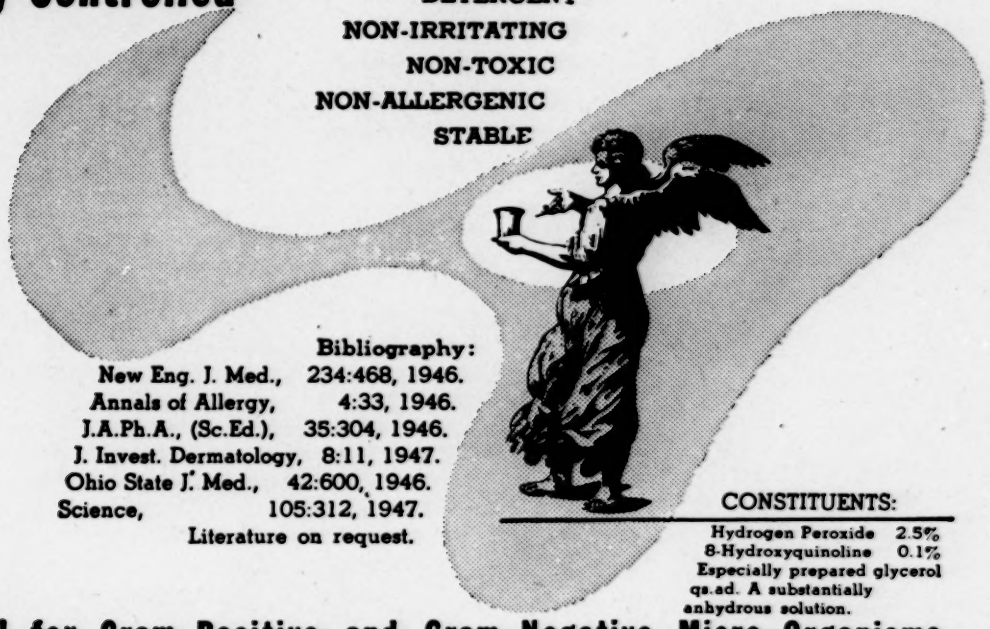
*Contributed by this magazine in co-operation with the Magazine Publishers of America as a public service.*

# INFECTIONS

## of the Skin and Mucous Membranes

**Rapidly Controlled**

BACTERICIDAL
HYGROSCOPIC
HEMOSTATIC
DETERGENT
NON-IRRITATING
NON-TOXIC
NON-ALLERGENIC
STABLE



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Literature on request.

CONSTITUENTS:

Hydrogen Peroxide 2.5%
8-Hydroxyquinoline 0.1%
Especially prepared glycerol qs.ad. A substantially anhydrous solution.

**Bactericidal for Gram-Positive and Gram-Negative Micro-Organisms**

# Glycerite of Hydrogen Peroxide *ipc*

**Treatment with Glycerite of Hydrogen Peroxide ipc Resulted in Rapid Control of the Infection in Conditions Diagnosed Clinically as:**

*vesicular, squamous, pustular dermatophytosis; onychomycosis; paronychiae; erosio interdigitale; aphthous stomatitis; herpes simplex; gingivitis; tonsillitis; epidermolysis bullosa; impetigo; varicose and diabetic ulcers; infected traumatic lesions of the skin and mucous membranes.*

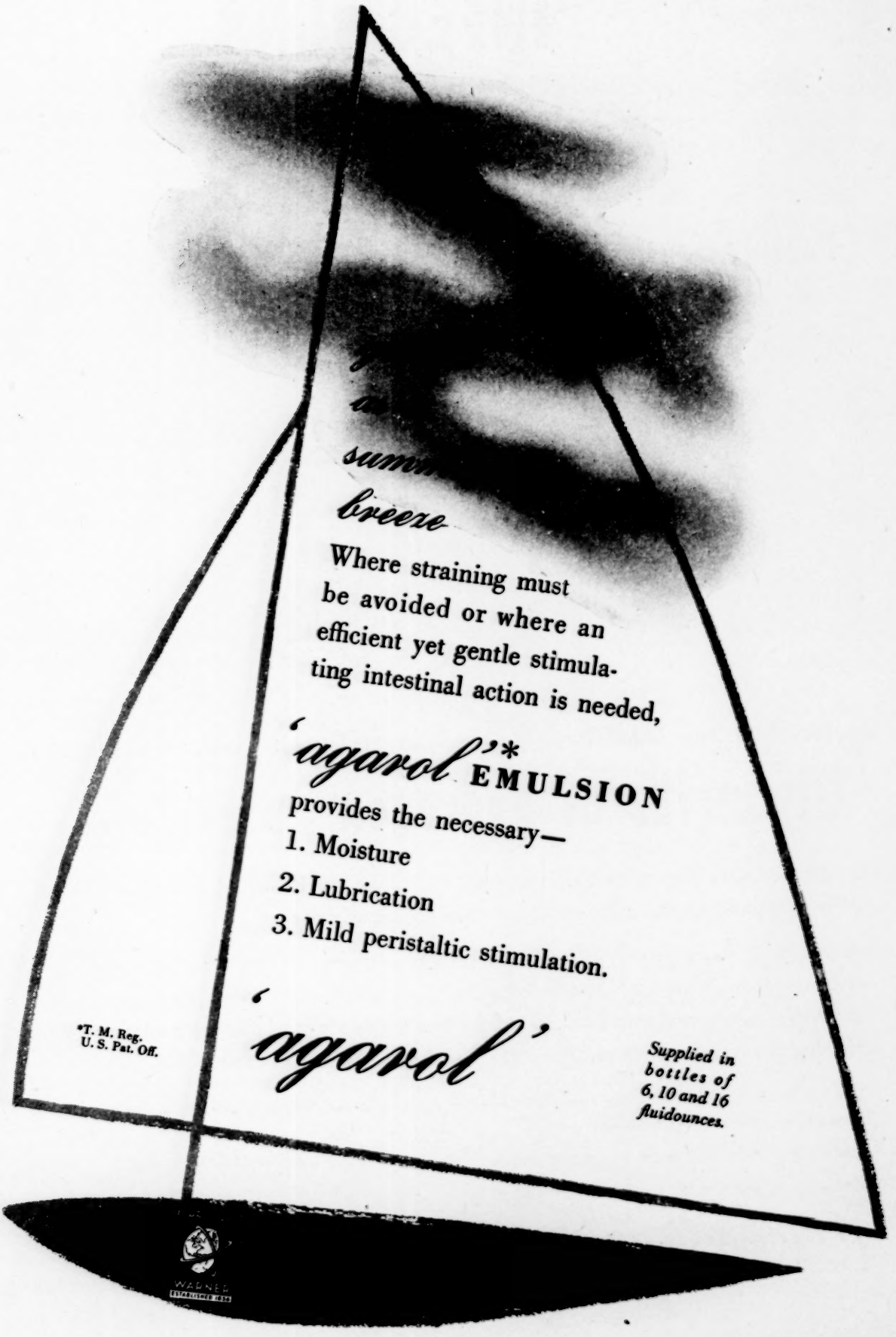
**Use full strength prophylactically as a post-operative application and therapeutically as a wet dressing renewed as frequently as indicated.**

**Use orally, diluted with water, as rinse or gargle.**

**Available on prescription in four-ounce bottles.**

*International* **PHARMACEUTICAL CORPORATION**

**132 Newbury Street, Boston 16, Massachusetts**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Where straining must be avoided or where an efficient yet gentle stimulating intestinal action is needed,
'agarol'* EMULSION
provides the necessary—
1. Moisture
2. Lubrication
3. Mild peristaltic stimulation.
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*T. M. Reg. U. S. Pat. Off.
Supplied in bottles of 6, 10 and 16 fluidounces.
WARNER